Electronic Supplementary Material (ESI) for Analyst.

Cationization of neutral small molecules by site-specific carboxylation of 10-phenyl-10H-phenothiazine in laser desorption/ionization

Chongqing Ma[†a], Tianrong Yu[†a], Yue Liu[†a], Rui Shi [a], Rui Lv [a], Ruochen Guo [a], Qinghua Cao [a], Gaole Dai [a], Yu Zhao *[a], Jian Liu *[a]

Institute of Functional Nano and Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou, Jiangsu Province 215123, China. Email: jliu@suda.edu.cn, yuzhao@suda.edu.cn

[†] These authors contributed to the manuscript equally.

Index

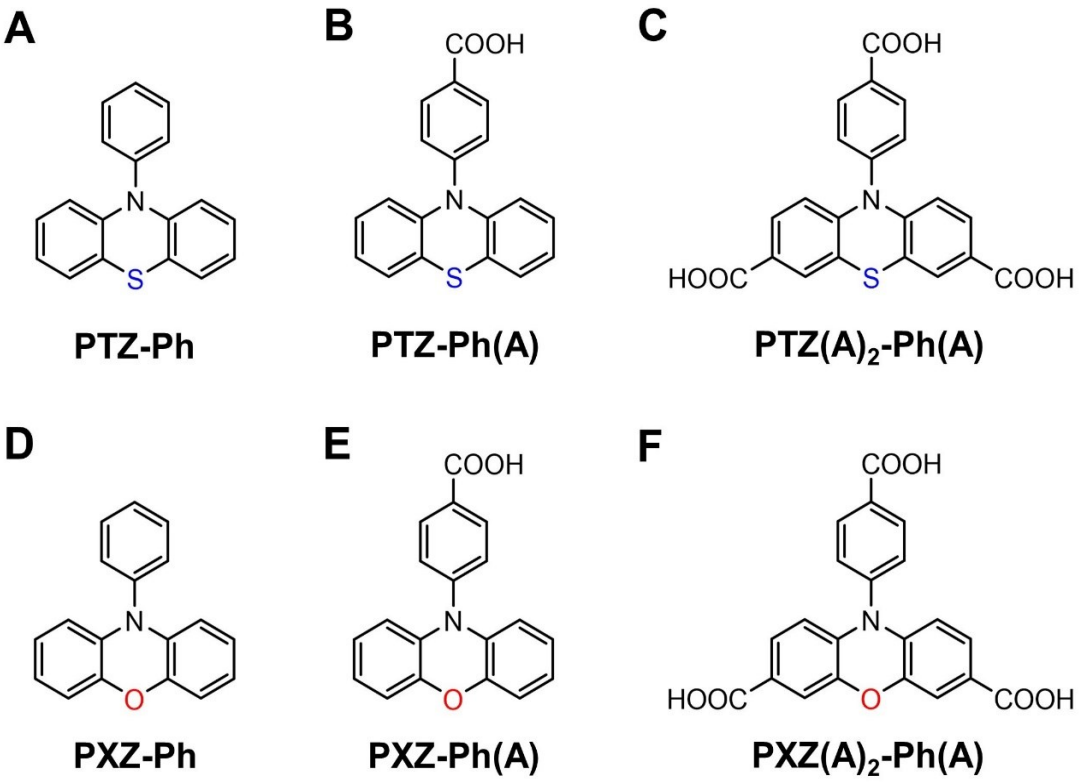


Figure S1. The chemical structures of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A) and $PXZ(A)_2$-Ph(A).

$$Na^+ + Cl^- \rightarrow NaCl \quad (1)$$

ΔH= -134.31 kcal/mol; ΔG= -129.22 kcal/mol

$$Glu + Na^+ \rightarrow Glu{\cdot}Na^+ \quad (2)$$

ΔH= -45.61 kcal/mol; ΔG= -37.36 kcal/mol

$$(PTZ - Ph)^{\cdot+} + Cl^- \rightarrow (PTZ - Ph)^{\cdot+} Cl^- \quad (3)$$

ΔH= -91.81 kcal/mol; ΔG= -85.02 kcal/mol

Scheme S1. The calculated enthalpy changes (*ΔH*) and Gibbs free energy changes (*ΔG*) of the reactions related to the cationization of GLC.

FT-IR

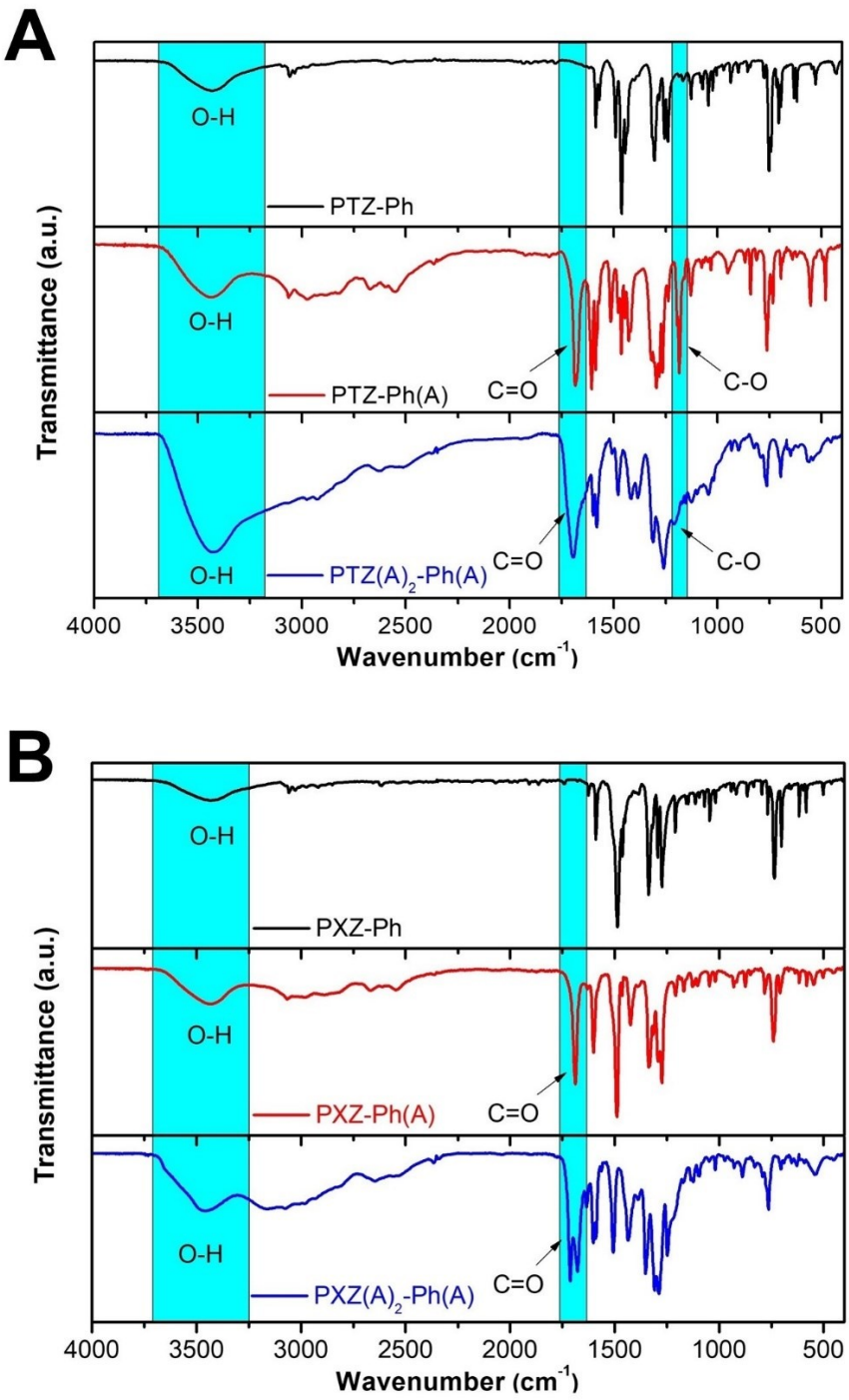


Figure S2. FT-IR spectra of six matrix candidates including PTZ-Ph, PTZ-Ph(A), PTZ(A)$_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), PXZ(A)$_2$-Ph(A).

UV-vis

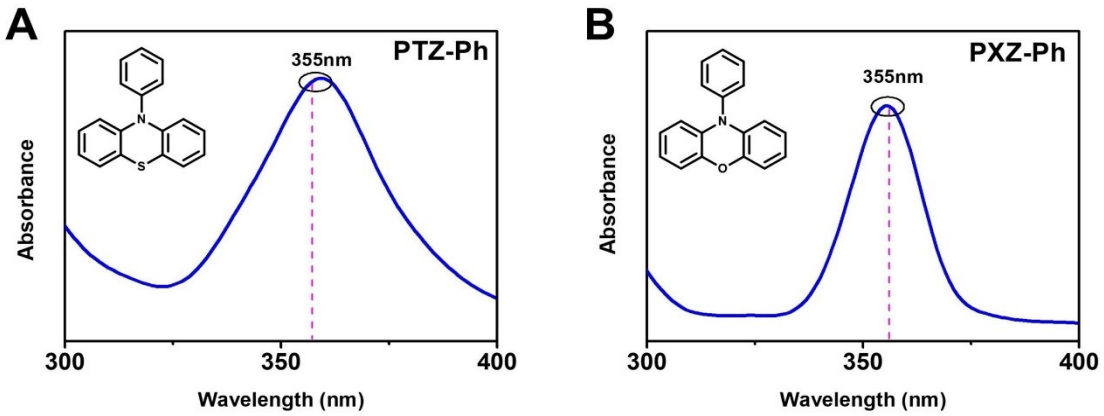


Figure S3. UV-vis absorption spectra of matrix candidates (A) PTZ-Ph; (B) PXZ-Ph.

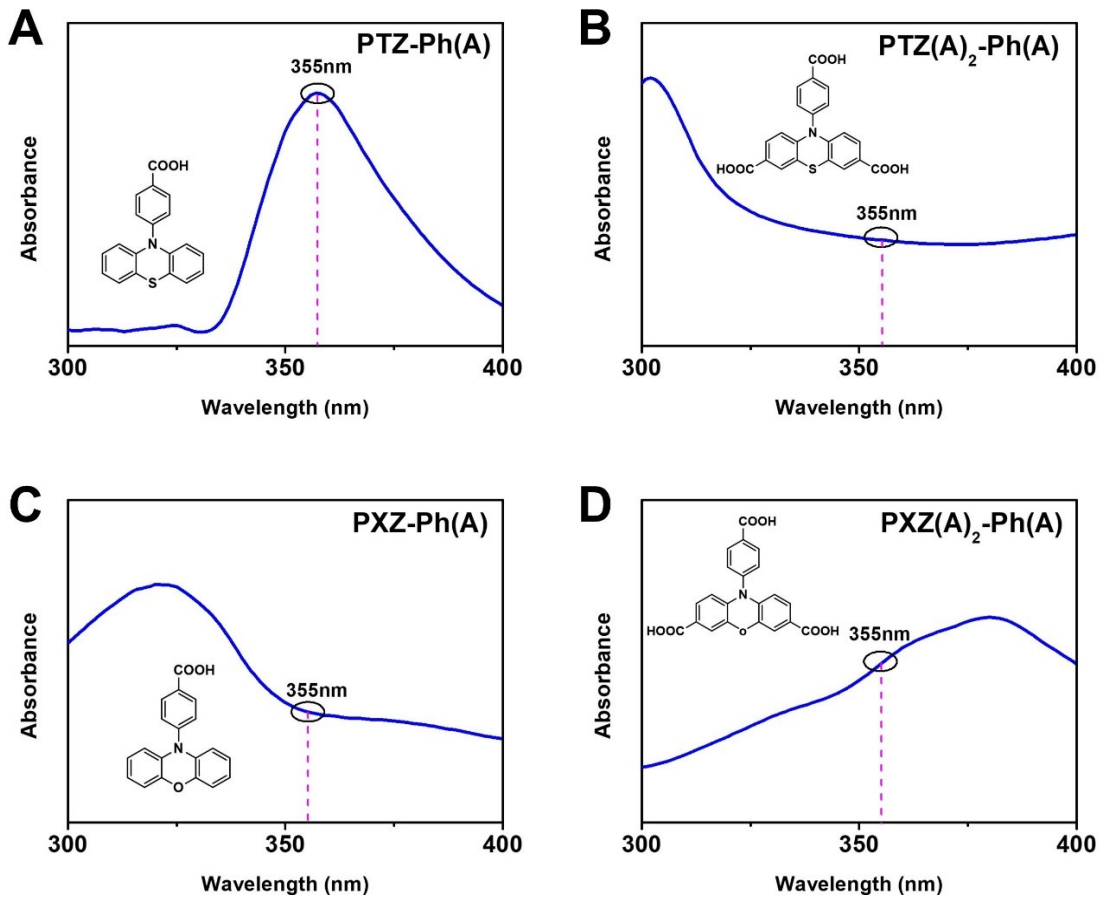
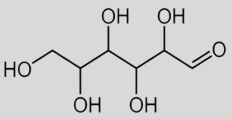
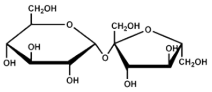
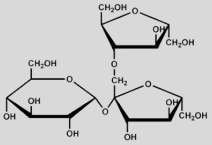
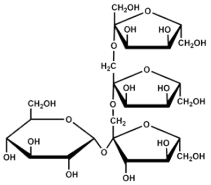
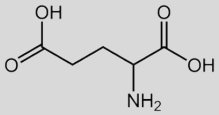
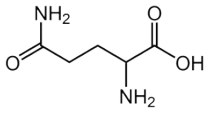
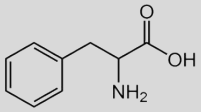
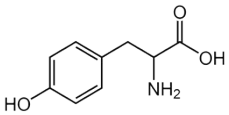
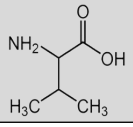


Figure S4. UV-vis absorption spectra of matrix candidates (A) PTZ-Ph(A), (B) $PTZ(A)_2$-Ph(A), (C) PXZ-Ph(A), (D) $PXZ(A)_2$-Ph(A).

Table S1. Summary of the analytes tested in this study.

Name	Abbrev.	Molecular formula	Molecular weight [g/mol]	Molecular structure
D-Glucose	GLC	$C_6H_{12}O_6$	180.16	
Sucrose	DP2	$C_{12}H_{22}O_{11}$	342.30	
Trisaccharide	DP3	$C_{18}H_{32}O_{16}$	504.44	
Tetrasaccharide	DP4	$C_{24}H_{42}O_{21}$	666.58	
L-Glutamine	Gln	$C_5H_{10}N_2O_3$	146.15	
L-Glutamic	Glu	$C_5H_9NO_4$	147.13	
L-Phenylalanine	Phe	$C_9H_{11}NO_2$	165.19	
L-Tyrosine	Tyr	$C_9H_{11}NO_3$	181.18	
L-Valine	Val	$C_5H_{11}NO_2$	117.15	

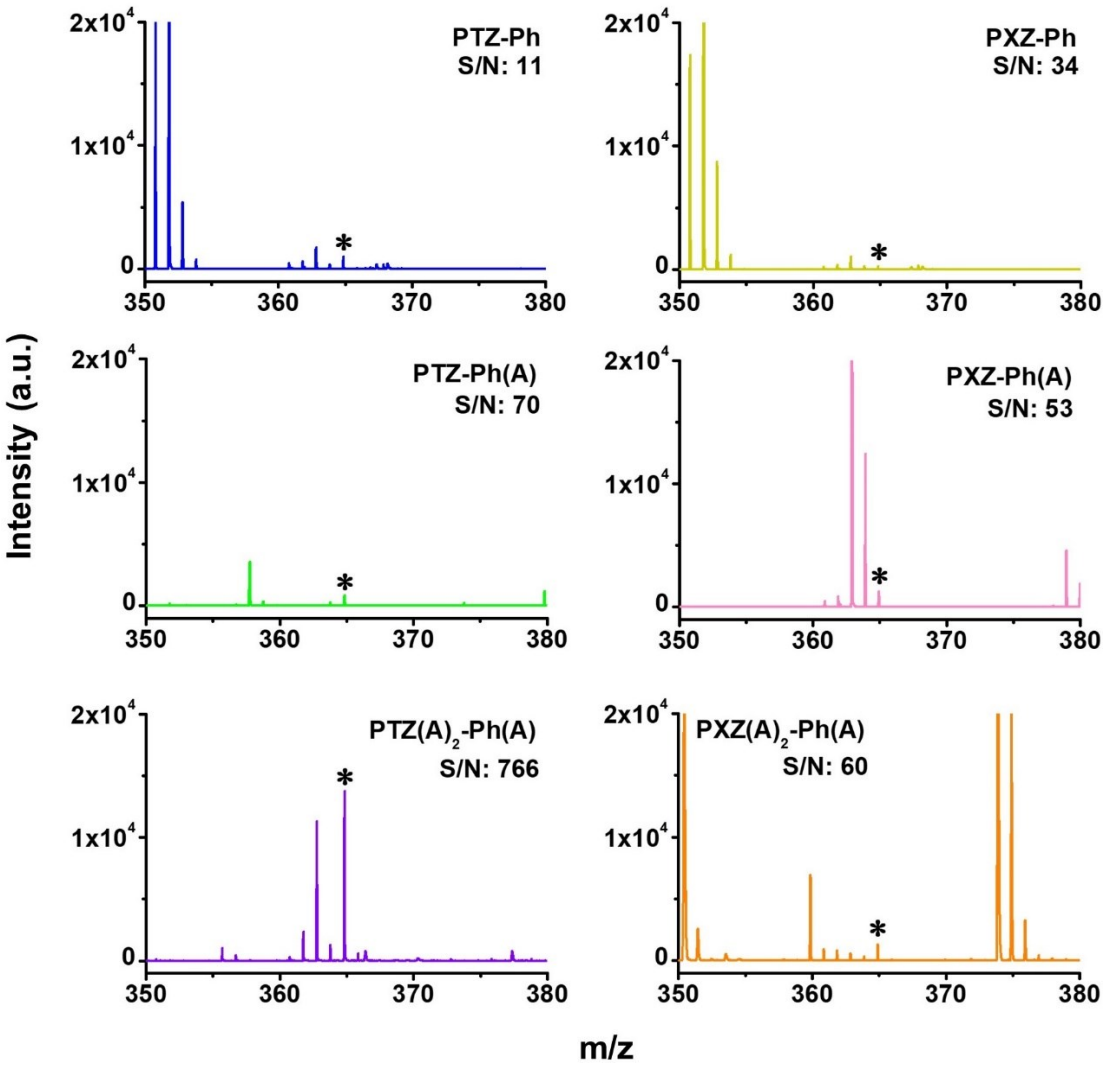


Figure S5. MALDI mass spectra of sucrose ($[M+Na]^+$, *m/z* 365.29) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. Sucrose concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

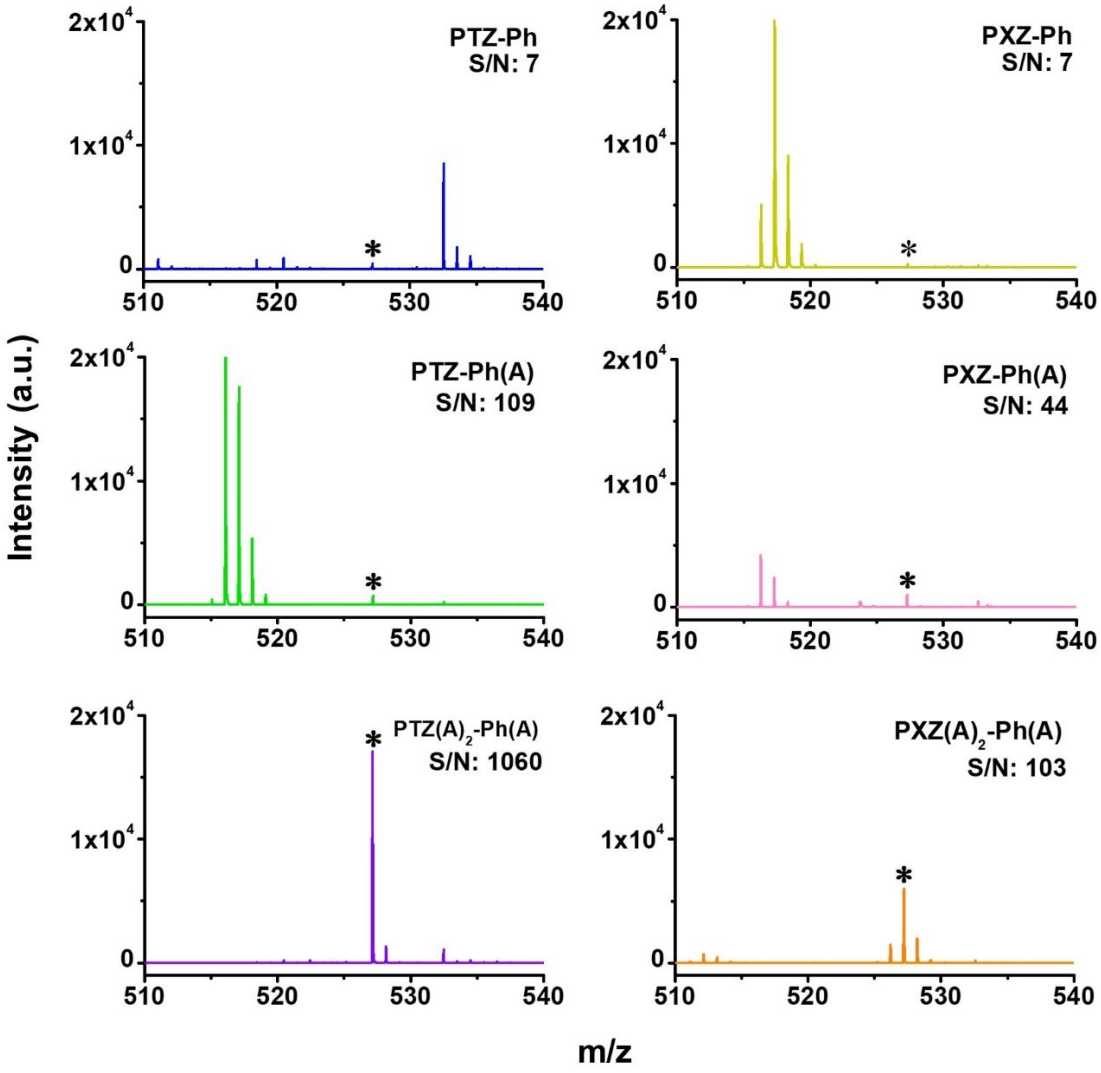


Figure S6. MALDI mass spectra of trisaccharide ($[M+Na]^+$, *m/z* 527.43) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. Trisaccharide concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

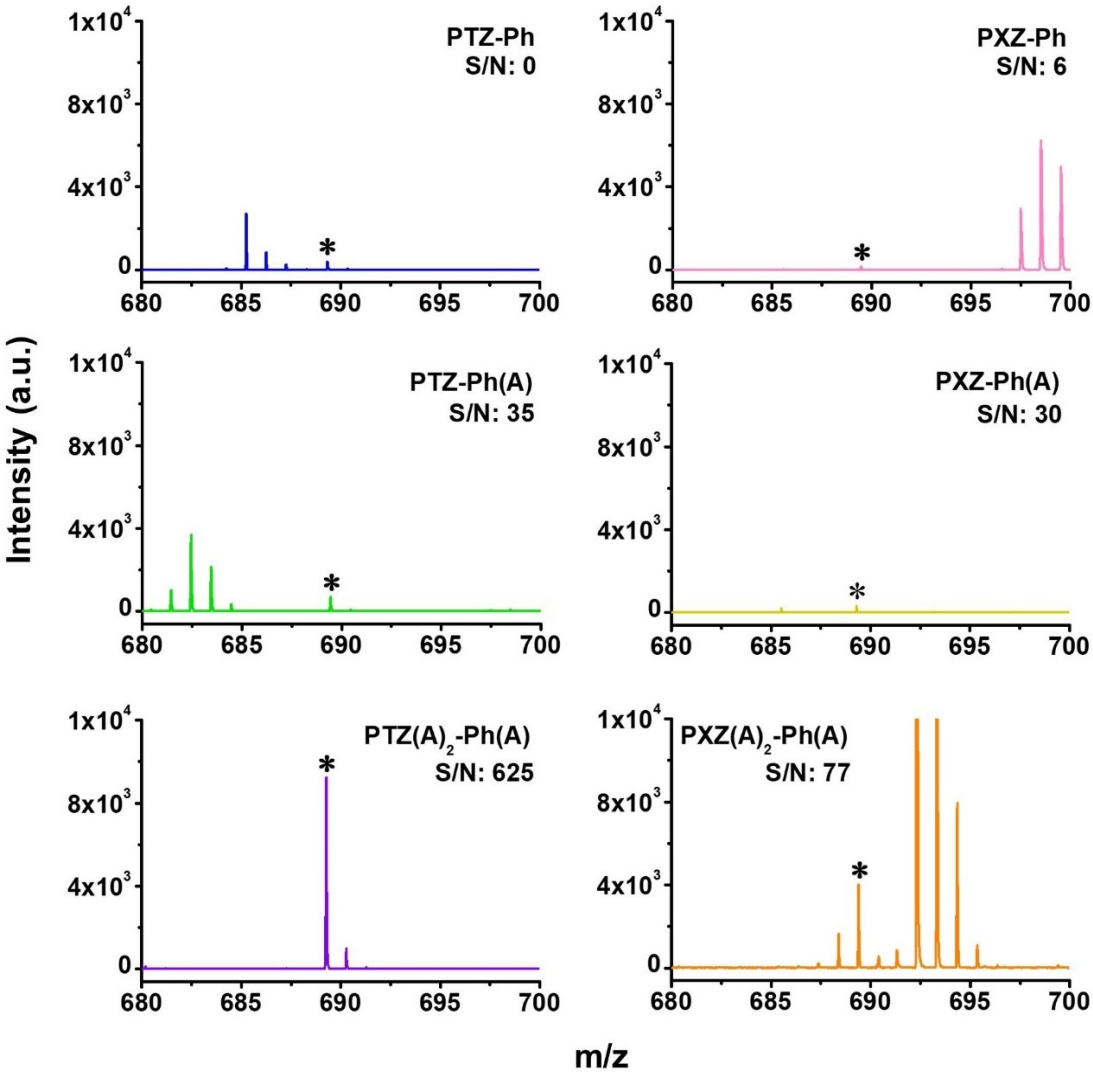


Figure S7. MALDI mass spectra of tetrasaccharide ($[M+Na]^+$, *m/z* 689.57) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. Tetrasaccharide concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

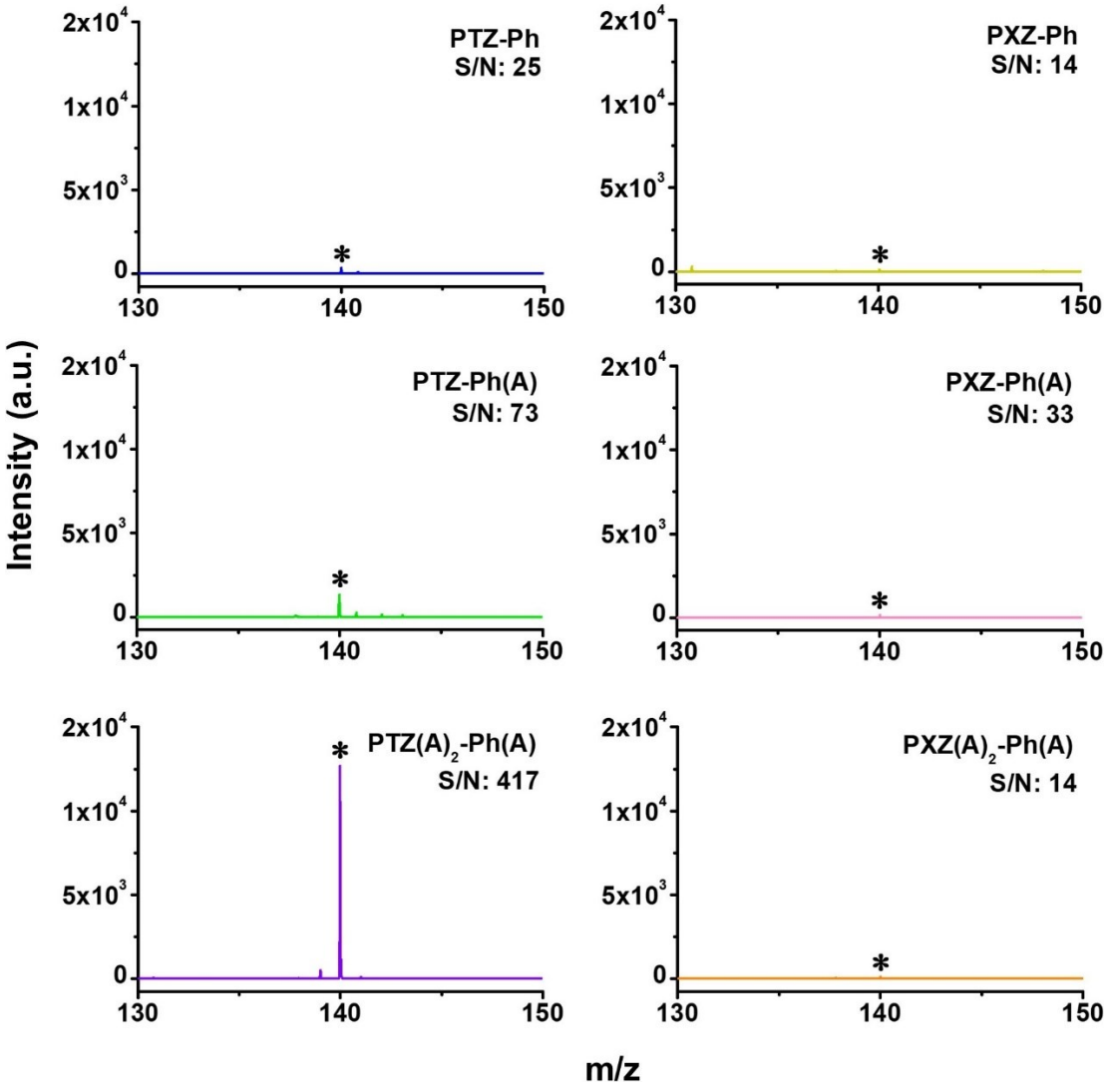


Figure S8. MALDI mass spectra of L-Valine ($[M+Na]^+$, *m/z* 140.14) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. L-Valine concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

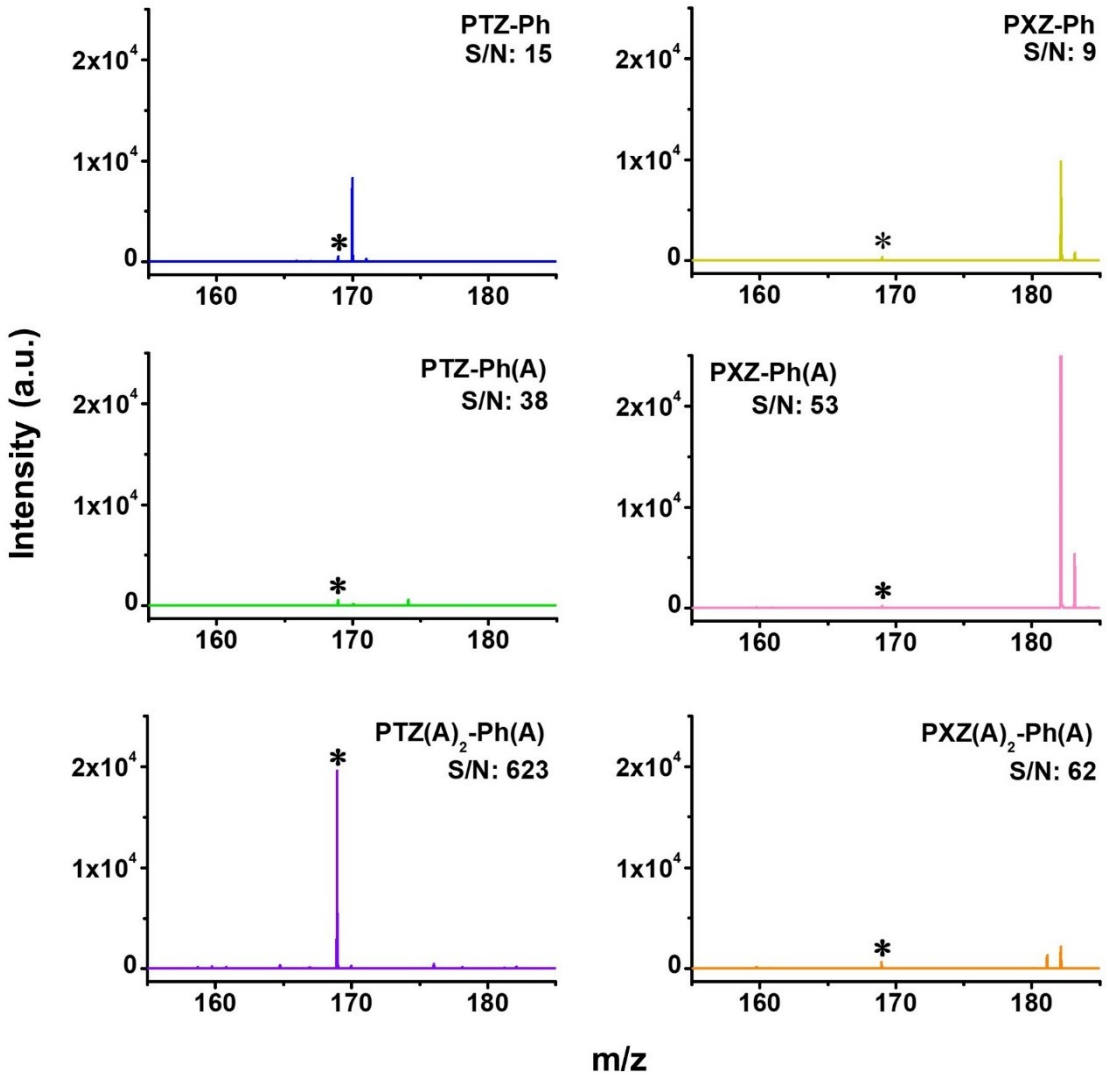


Figure S9. MALDI mass spectra of L-Glutamine ($[M+Na]^+$, *m/z* 169.14) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. L-Glutamine concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

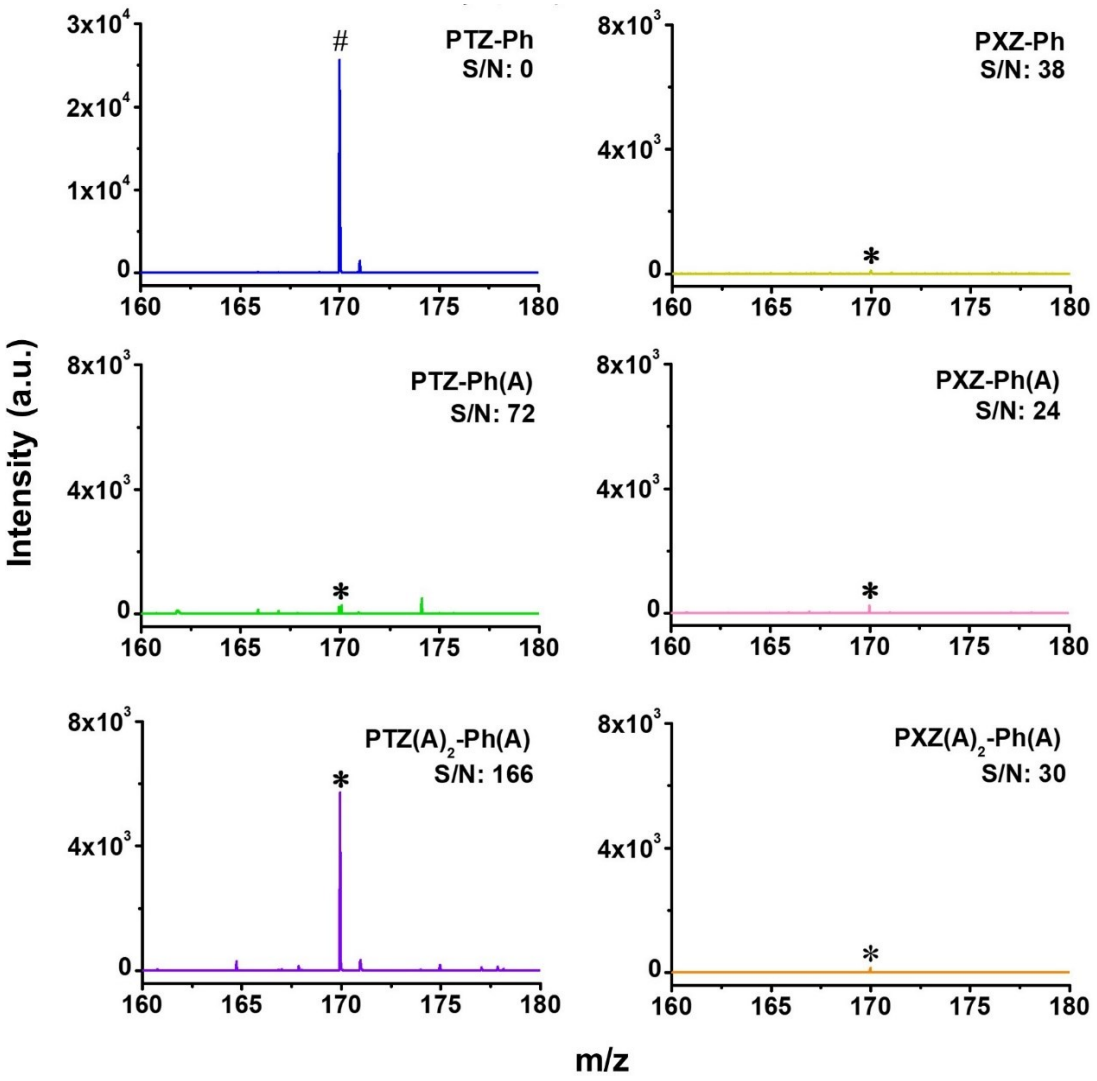


Figure S10. MALDI mass spectra of L-Glutamic ($[M+Na]^+$, *m/z* 170.12) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. L-Glutamic concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

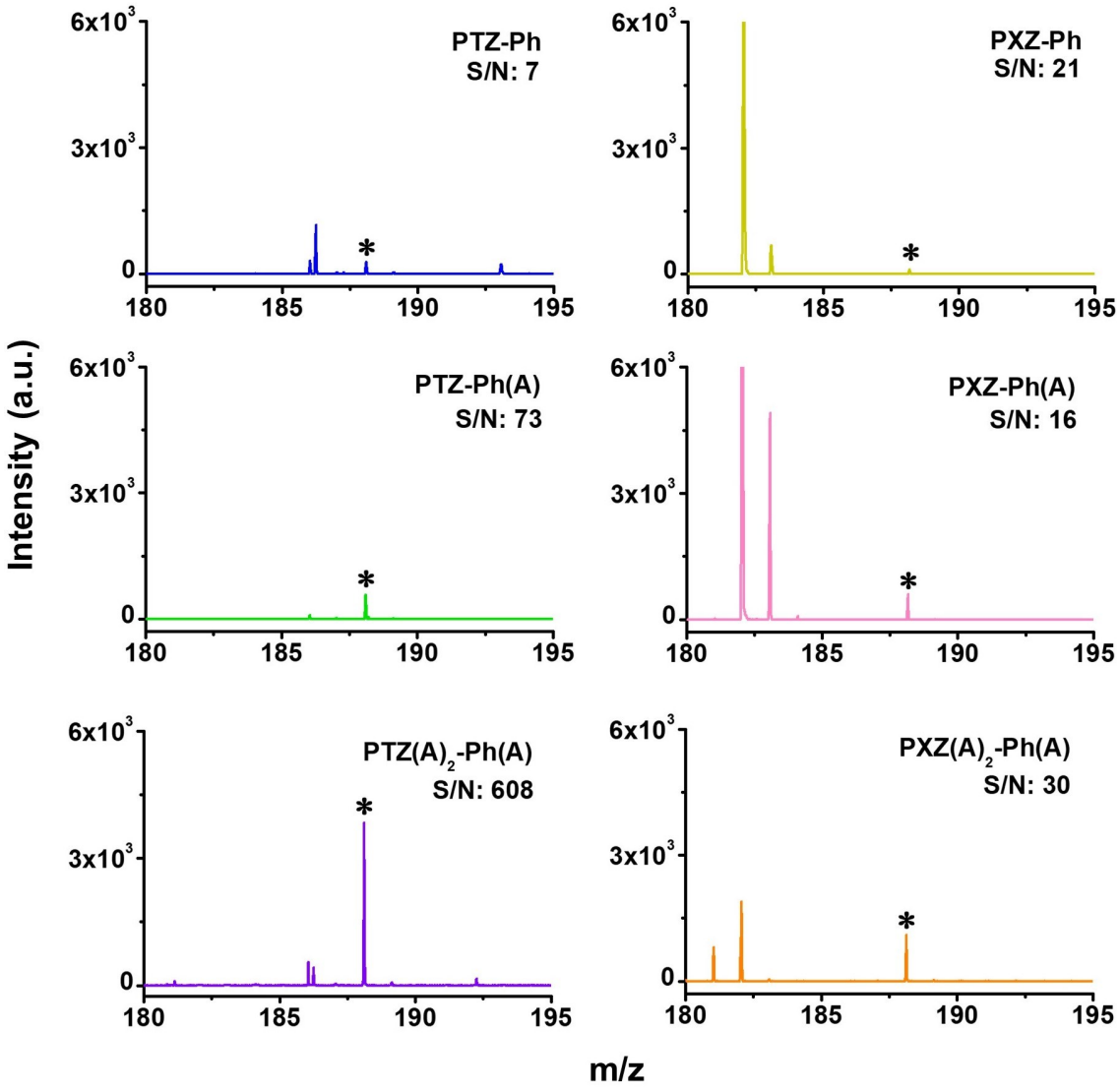


Figure S11. MALDI mass spectra of L-Phenylalanine ($[M+Na]^+$, *m/z* 188.18) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. L-Phenylalanine concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

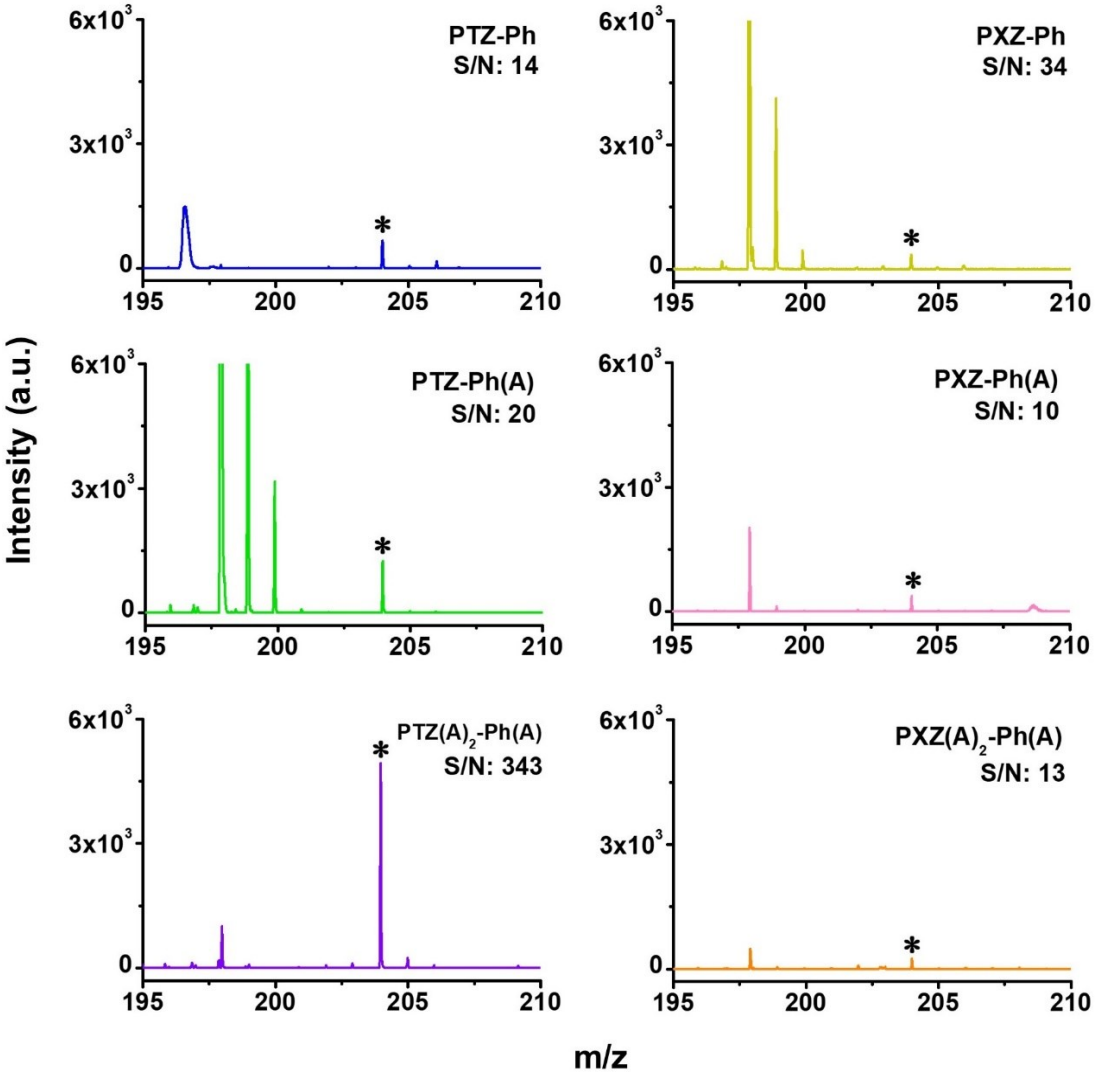


Figure S12. MALDI mass spectra of L-Tyrosine ($[M+Na]^+$, *m/z* 204.17) with the matrix candidates of PTZ-Ph, PTZ-Ph(A), $PTZ(A)_2$-Ph(A), PXZ-Ph, PXZ-Ph(A), $PXZ(A)_2$-Ph(A) in the positive-ion mode of MALDI MS. L-Tyrosine concentration: 100 μg/mL, laser intensity of 57 μJ, accumulated to 3000 shots.

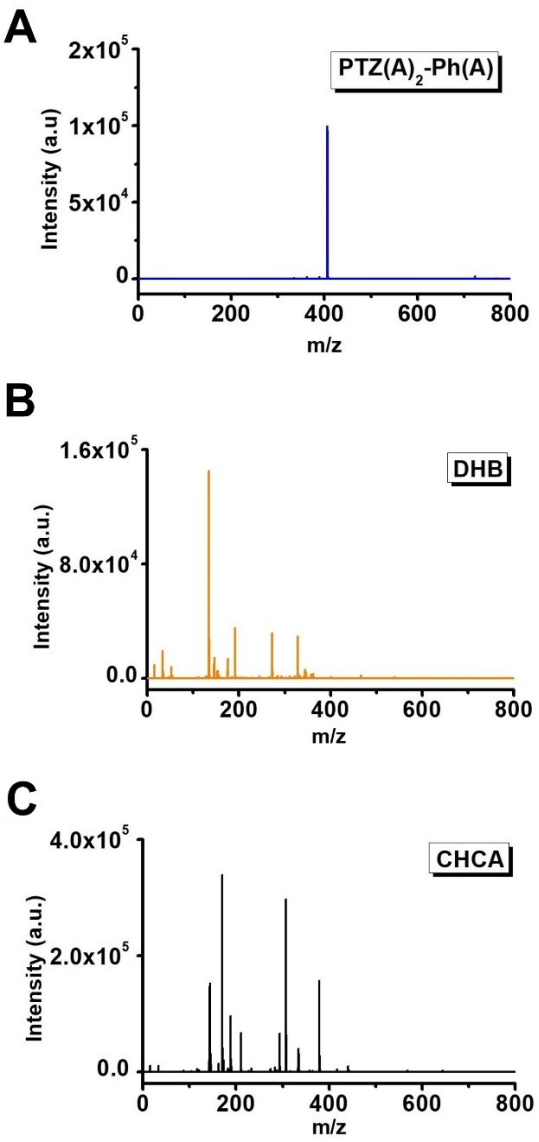


Figure S13. Comparison of background noise levels of matrices, including (A) PTZ(A)$_2$-Ph(A), (B) DHB, and (C) CHCA, in the positive-ion mode of MALDI MS. Display range: *m/z* 0-800.

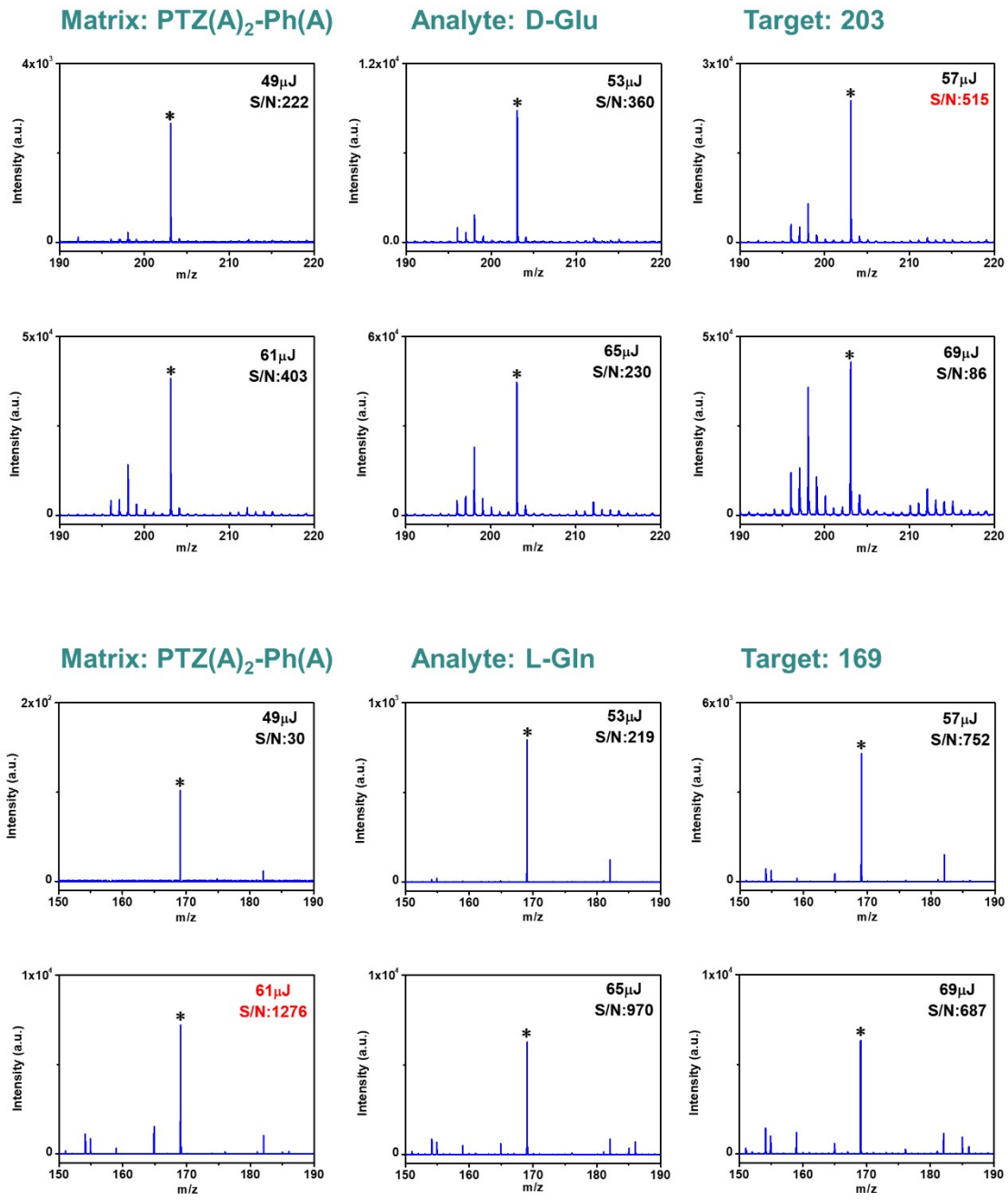


Figure S14. MALDI mass spectra of the D-Glucose (GLC) and L-Glutamine with PTZ(A)$_2$-Ph(A) matrix in the positive-ion mode. The laser pulse energy levels include 49 μJ, 53 μJ, 57 μJ, 61 μJ, 65 μJ and 69 μJ, respectively. The optimal laser energy is 57 μJ for D-Glucose (GLC), or 61 μJ for L-Glutamine (L-Gln). Accumulation: 2000 shots. Laser spot size: 50~100 μm.

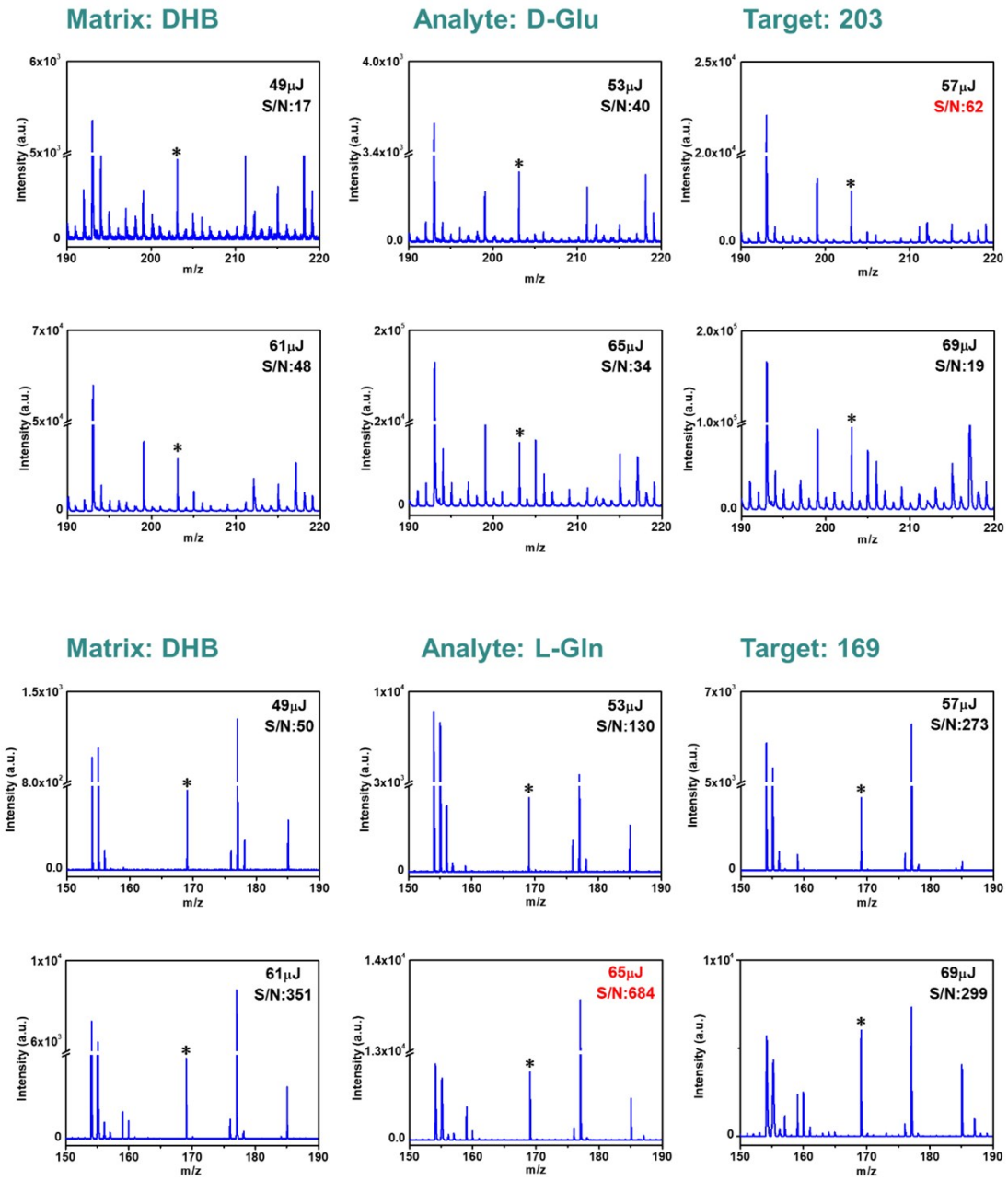


Figure S15. MALDI mass spectra of the D-Glucose (GLC) and L-Glutamine with DHB matrix in the positive-ion mode. The laser pulse energy levels include 49 μJ, 53 μJ, 57 μJ, 61 μJ, 65 μJ and 69 μJ, respectively. The optimal laser energy is 57 μJ for D-Glucose (GLC), or 65 μJ for L-Glutamine (L-Gln). Accumulation: 2000 shots. Laser spot size: 50~100 μm.

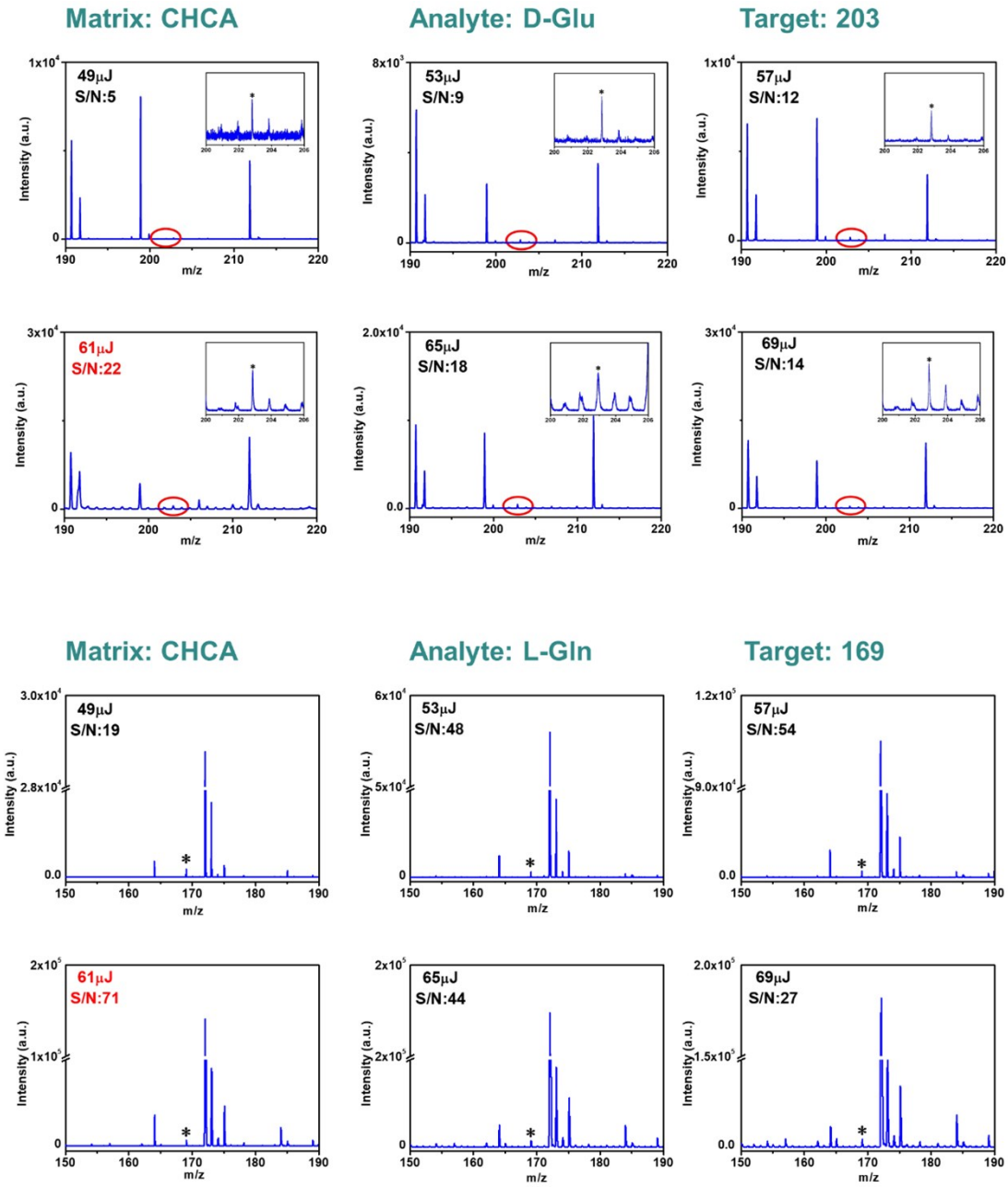
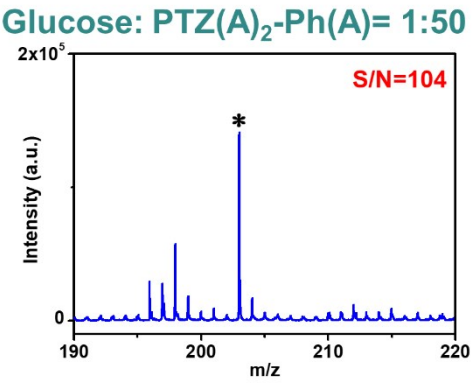


Figure S16. MALDI mass spectra of the D-Glucose (GLC) and L-Glutamine with CHCA matrix in the positive-ion mode. The laser pulse energy levels include 49 μJ, 53 μJ, 57 μJ, 61 μJ, 65 μJ and 69 μJ, respectively. The optimal laser energy is 61 μJ for D-Glucose (GLC) and L-Glutamine (L-Gln). Accumulation: 2000 shots. Laser spot size: 50~100 μm.

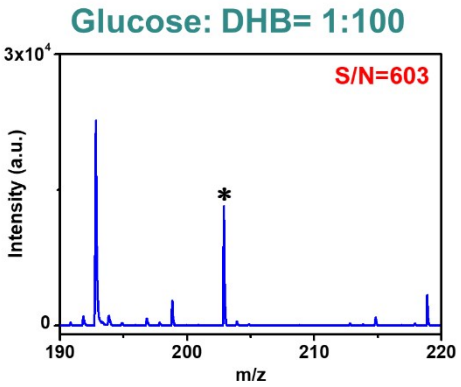
A

Matrix	Molecule Ratio Glucose / $PTZ(A)_2$-Ph(A)	S/N
$PTZ(A)_2$-Ph(A)	1:1000	8
	1:500	20
	1:250	40
	1:100	68
	1:50	104
	1:25	78



B

Matrix	Molecule Ratio Glucose / DHB	S/N
DHB	1:200	207
	1:100	603
	1:50	434
	1:20	156
	1:10	113
	1:5	0



C

Matrix	Molecule Ratio Glucose / CHCA	S/N
CHCA	1:100	8
	1:50	19
	1:25	26
	1:10	41
	1:5	58
	1:2.5	112

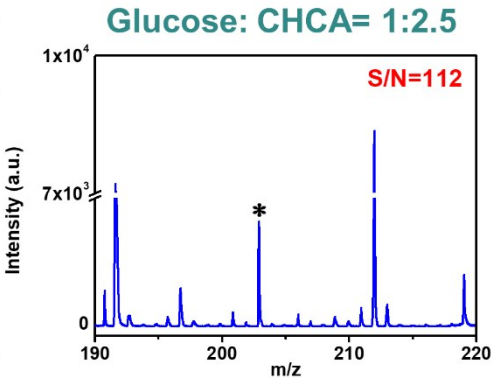
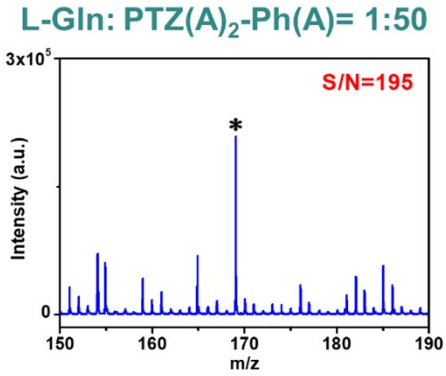


Figure S17. The optimal results of mixing ratio of analyte (GLC) and matrix ($PTZ(A)_2$-Ph(A), DHB and CHCA) in the positive-ion mode. Different mixing ratios and signal to instrumental noise ratios are listed in the table (left). The MS spectrum at the optimal mixing ratio is presented by the graph (right). The optimal mixing molecule ratio for the D-Glucose (GLC) using $PTZ(A)_2$-Ph(A), DHB and CHCA is 1:50, 1:100 and 1:2.5, respectively. Accumulation: 2000 shots. Laser spot size: 50~100 μm.

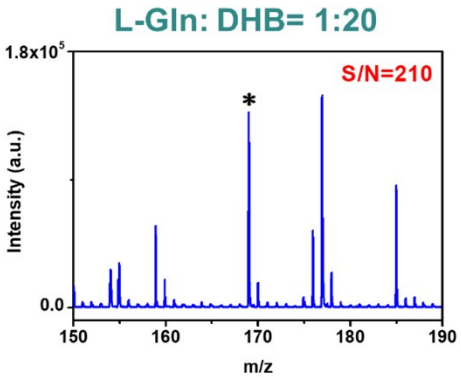
A

Matrix	Molecule Ratio L-Gln / $PTZ(A)_2$-Ph(A)	S/N
$PTZ(A)_2$-Ph(A)	1:1000	12
	1:500	23
	1:250	58
	1:100	103
	1:50	195
	1:25	138



B

Matrix	Molecule Ratio L-Gln / DHB	S/N
DHB	1:400	66
	1:200	81
	1:100	95
	1:40	123
	1:20	210
	1:10	155



C

Matrix	Molecule Ratio L-Gln / CHCA	S/N
CHCA	1:400	38
	1:200	160
	1:100	182
	1:40	91
	1:20	86
	1:10	59

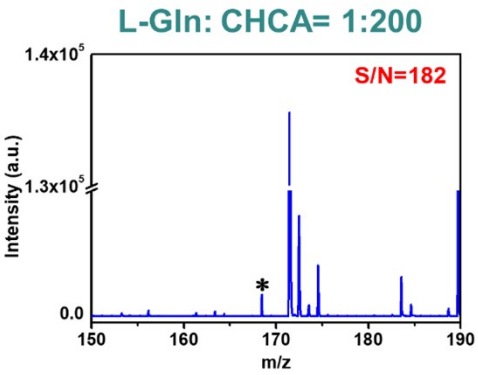


Figure S18. The optimal results of mixing ratio of analyte (L-Glutamine) and matrix ($PTZ(A)_2$-Ph(A), DHB and CHCA) in the positive-ion mode. Different mixing ratios and signal to instrumental noise ratios are listed in the table (left). The MS spectrum at the optimal mixing ratio is presented by the graph (right). The optimal mixing molecule ratio for the L-Glutamine using $PTZ(A)_2$-Ph(A), DHB and CHCA is 1:50, 1:100 and 1:2.5, respectively. Accumulation: 2000 shots. Laser spot size: 50~100 μm.

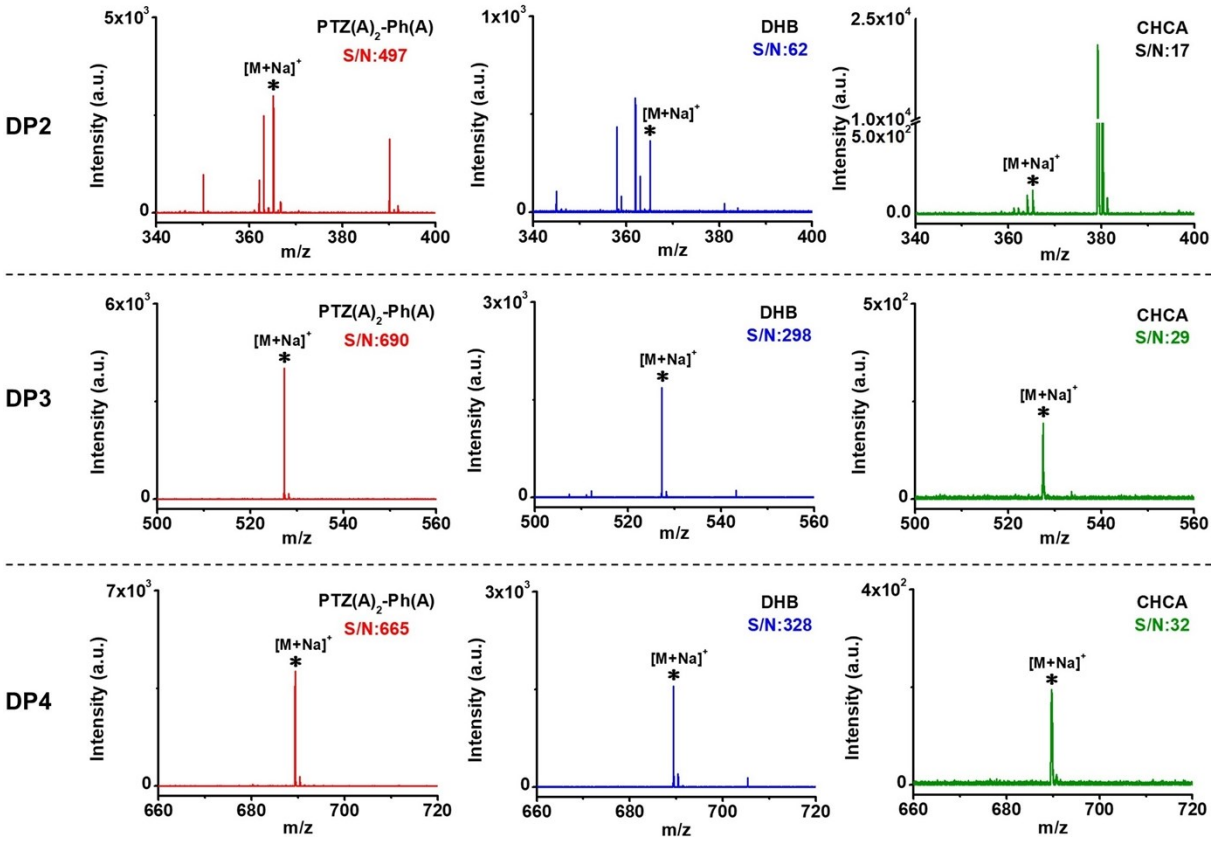


Figure S19. MALDI mass spectra of Sucrose (DP2), Trisaccharide (DP3) and Tetrasaccharide (DP4) obtained from the PTZ(A)$_2$-Ph(A) matrix and the traditional matrixes of DHB and CHCA. All analytes are tested in the concentration of 100 μg/mL.

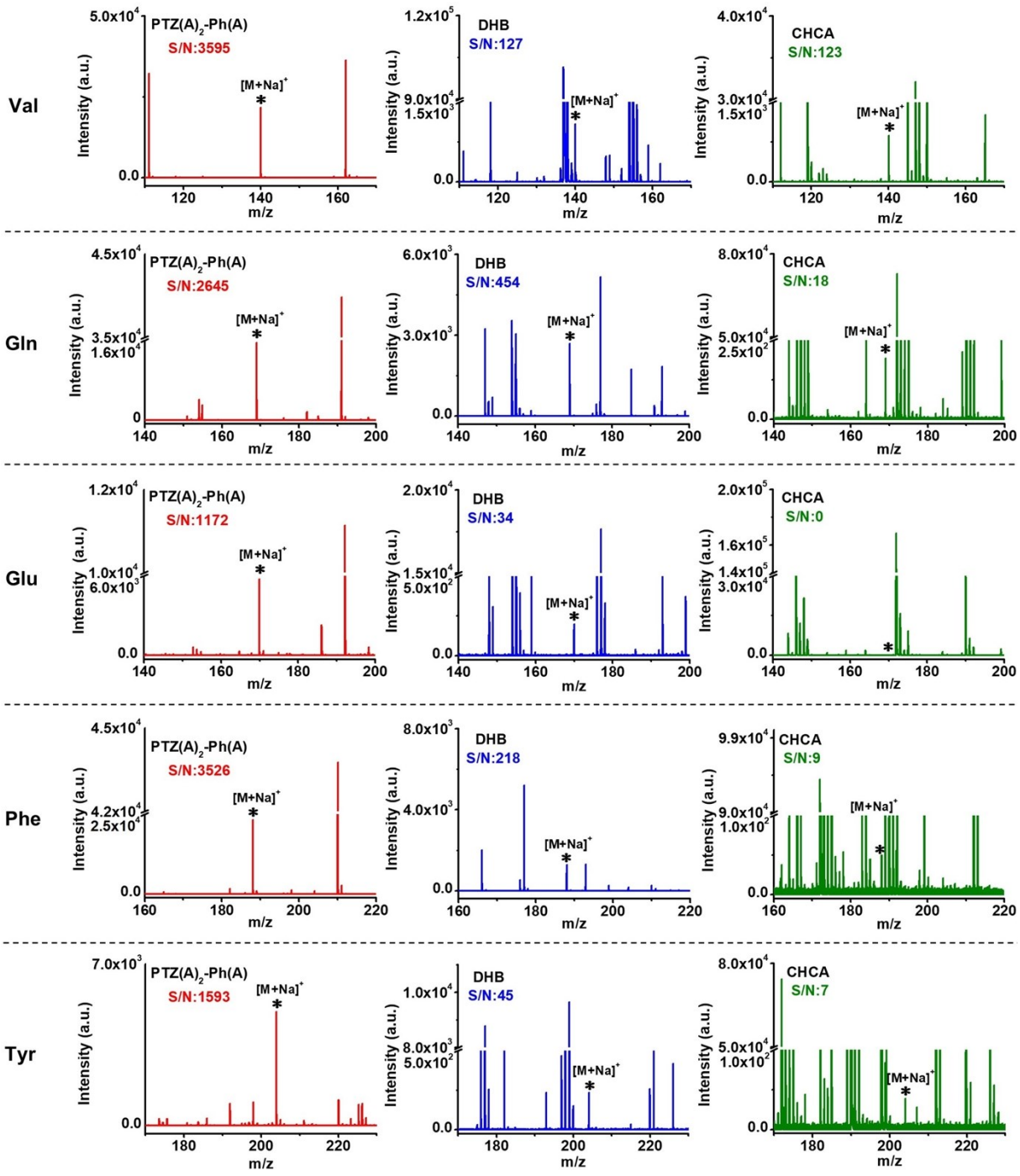


Figure S20. MALDI mass spectra of L-Valine (Val), L-Glutamine (Gln), L-Glutamic (Glu), L-Phenylalanine (Phe) and L-Tyrosine (Tyr) obtained from the $PTZ(A)_2$-Ph(A) matrix and the traditional matrixes of DHB and CHCA. All analytes are tested in the concentration of 100 μg/mL.

Table S2. Comparison of signal to noise (S/N) ratios for selected oligosaccharides in cationization ionization mechanisms with PTZ(A)$_2$-Ph(A), DHB and CHCA matrixes.

Matrix	**PTZ(A)$_2$-Ph(A)**	**DHB**	**CHCA**
Ion peak / Analyte	**[M+Na]$^+$**	**[M+Na]$^+$**	**[M+Na]$^+$**
GLC	362	48	17
DP2	497	62	17
DP3	690	298	29
DP4	665	328	32

Table S3. Comparison of signal to noise (S/N) ratios for selected amino acids in different ionization mechanisms with PTZ(A)$_2$-Ph(A), DHB and CHCA matrixes.

Matrix	**PTZ(A)$_2$-Ph(A)**	**DHB**		**CHCA**	
Ion peak / Analyte	**[M+Na]$^+$**	**[M+Na]$^+$**	[M+H]$^+$	**[M+Na]$^+$**	[M+H]$^+$
Val	**3595**	**127**	307	**123**	1502
Gln	**2645**	**454**	510	**18**	1471
Glu	**1172**	**34**	106	**0**	714
Phe	**3526**	**218**	676	**9**	1340
Tyr	**1593**	**45**	61	**7**	1251

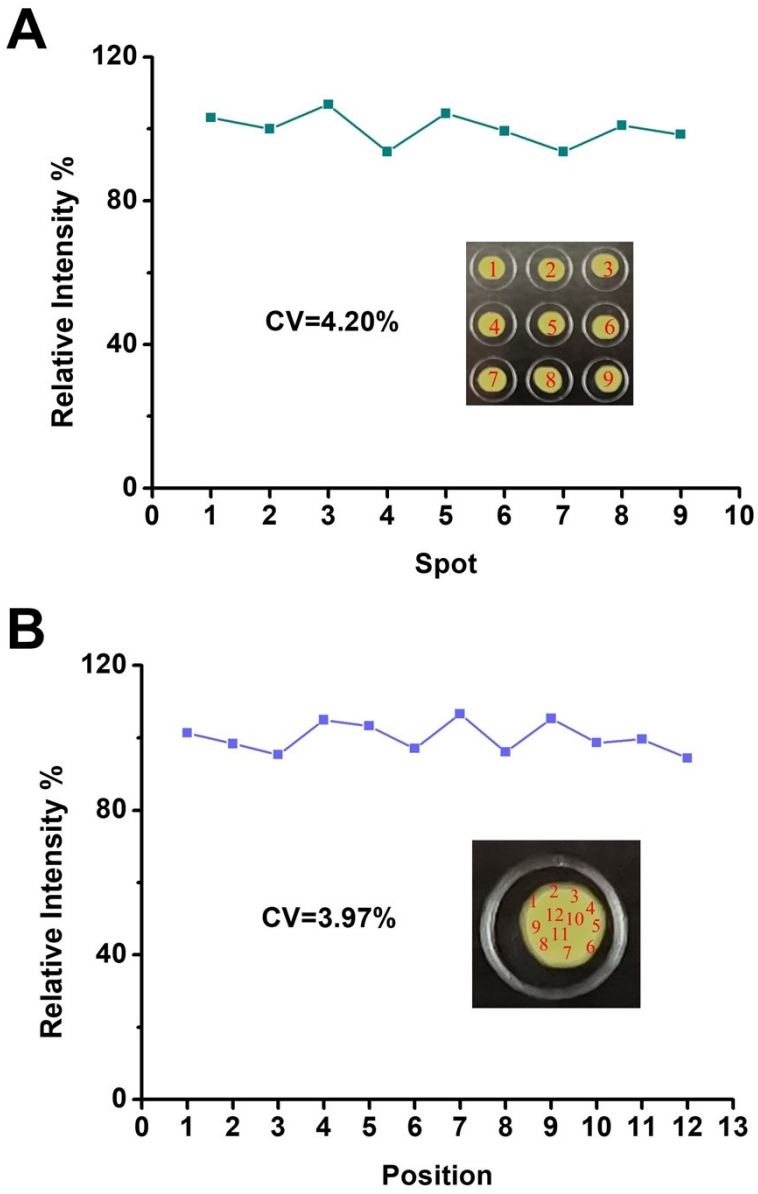


Figure S21. The PTZ(A)$_2$-Ph(A) matrix performed highly reproducible MS signal intensity. (A) 9 different spots in the 3 × 3 array. (B) 12 different positions in a single spot of GLC/ PTZ(A)$_2$-Ph(A). The coefficient of variation (CV) values as very small, only (A) 4.20 % in the spot-to-spot or (B) 3.97 % in the position-to-position. Inset: The Photo of the PTZ(A)$_2$-Ph(A) as the matrix dropped on the steel plate, forming uniform films. Analyte: D-glucose (GLC), concentration: 100 μg/mL.

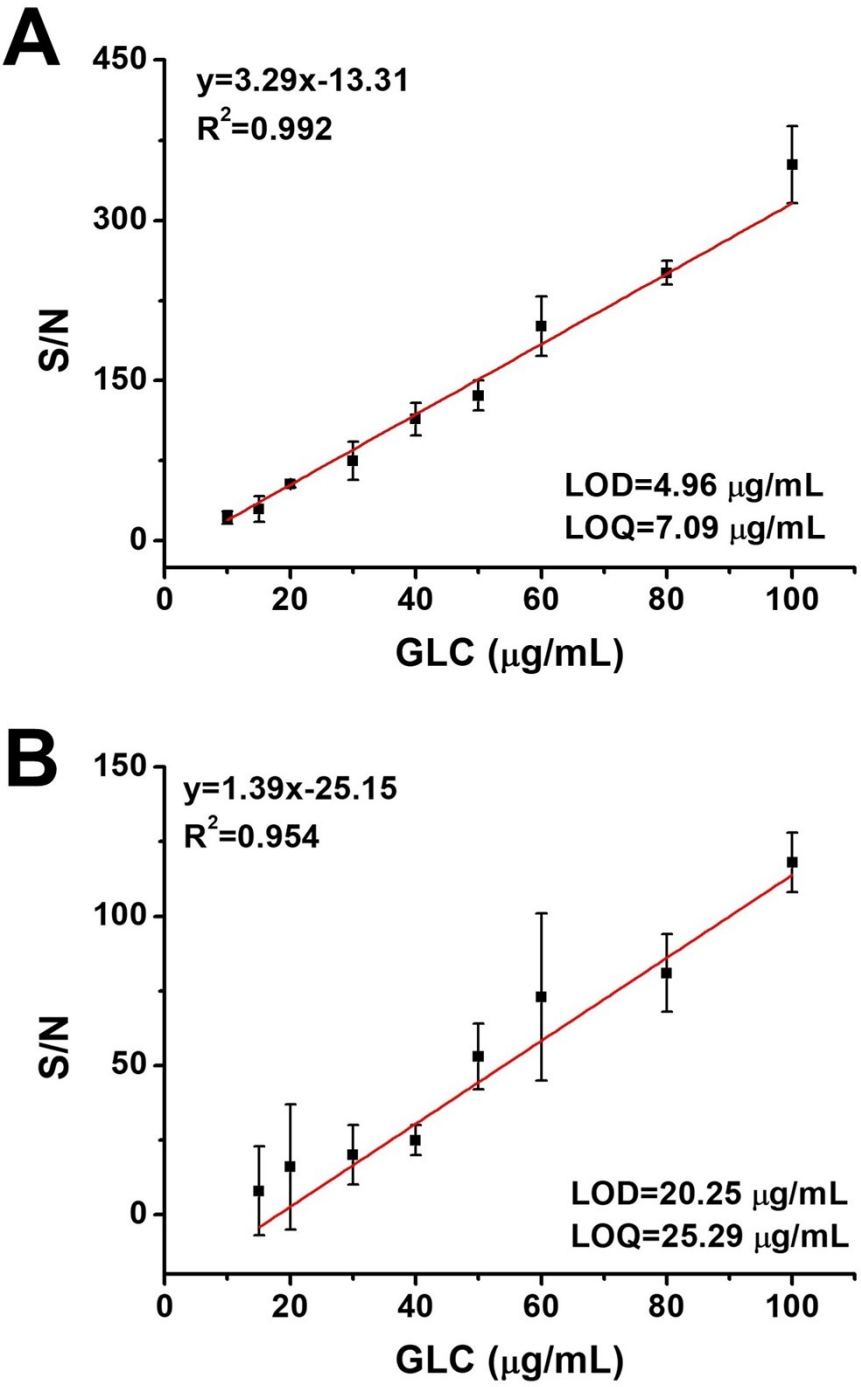


Figure S22. The quantitative analysis of linearity and limit-of-detection for the $PTZ(A)_2$-Ph(A) matrix. MALDI-TOF MS response curves for different concentrations glucose (GLC) with (A) $PTZ(A)_2$-Ph(A) matrix and (C) DHB matrix. Accumulation: 3000 shots. Laser spot size: 50~100 μm.

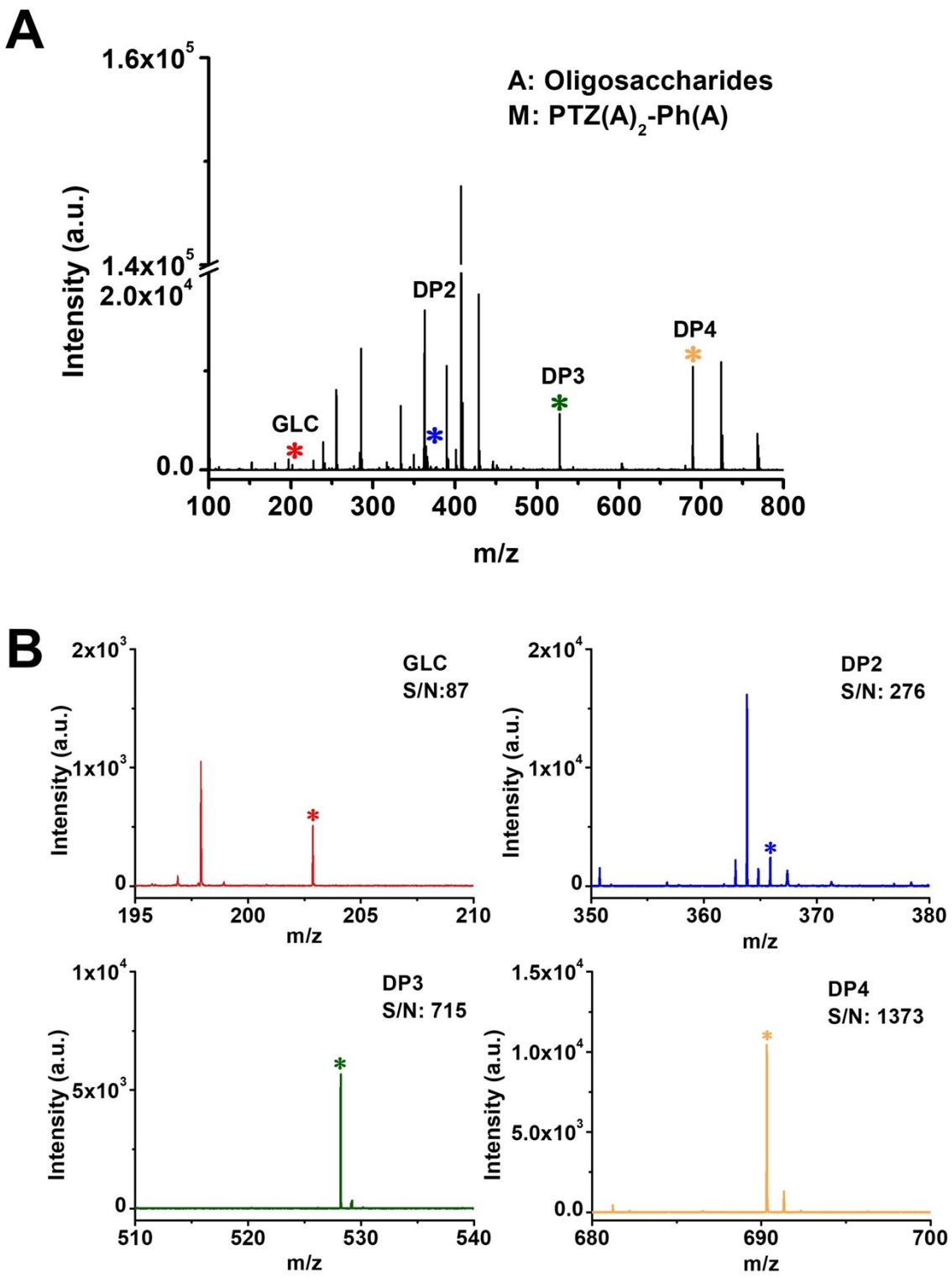


Figure S23. (A) MALDI-TOF mass spectra of a complex sample containing four oligosaccharides with the $PTZ(A)_2$-Ph(A) matrix. (B) MALDI-TOF mass spectra and signal-to-noise ratio (S/N) of GLC, DP2, DP3 and DP4 in complex sample detection with the $PTZ(A)_2$-Ph(A) matrix. The concentrations of all analytes are 250 μg/mL.

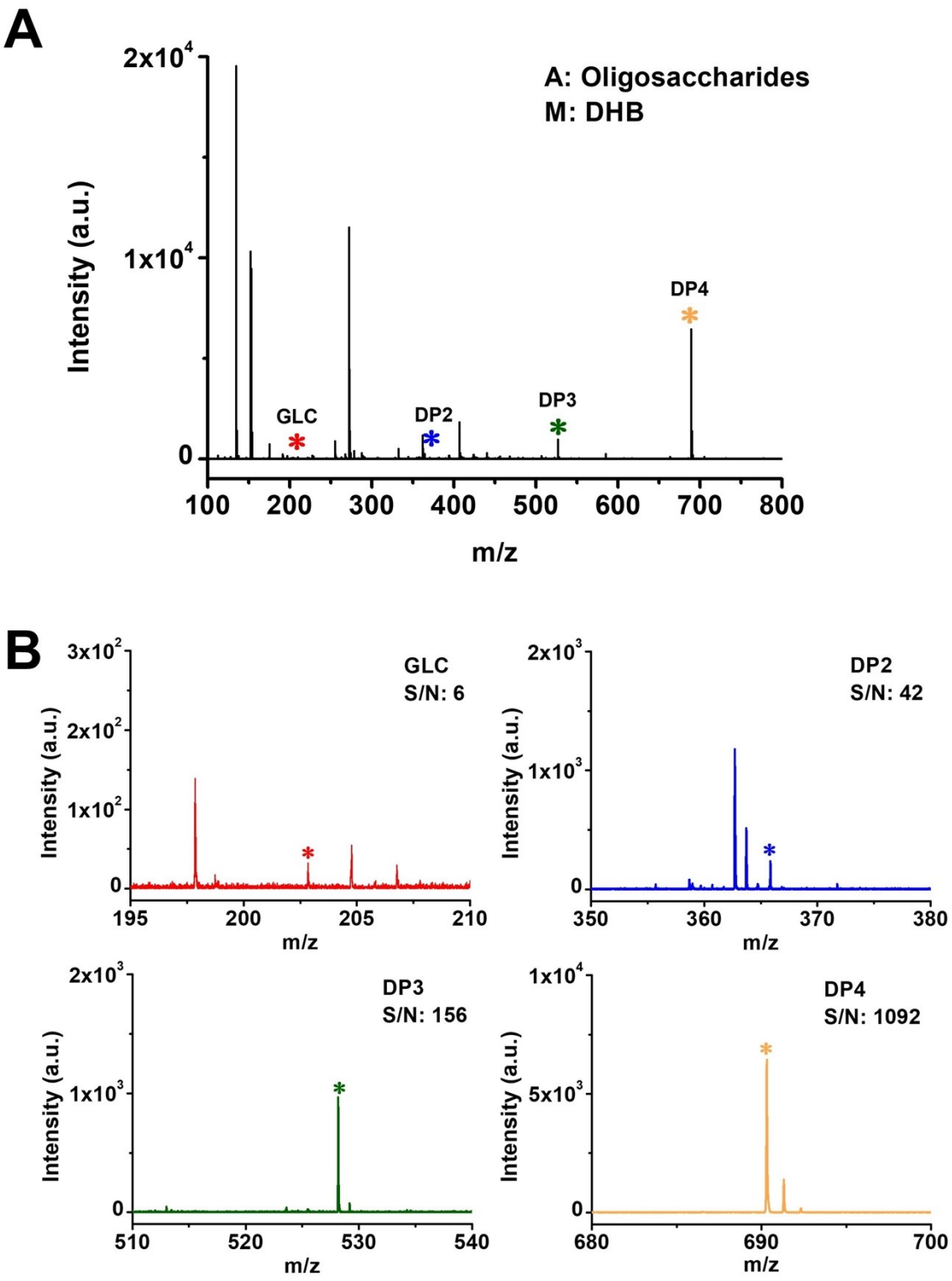


Figure S24. (A) MALDI-TOF mass spectra of a complex sample containing four oligosaccharides with the DHB matrix. (B) MALDI-TOF mass spectra and signal-to-noise ratio (S/N) of GLC, DP2, DP3 and DP4 in complex sample detection with the DHB matrix. The concentrations of all analytes are 250 μg/mL.

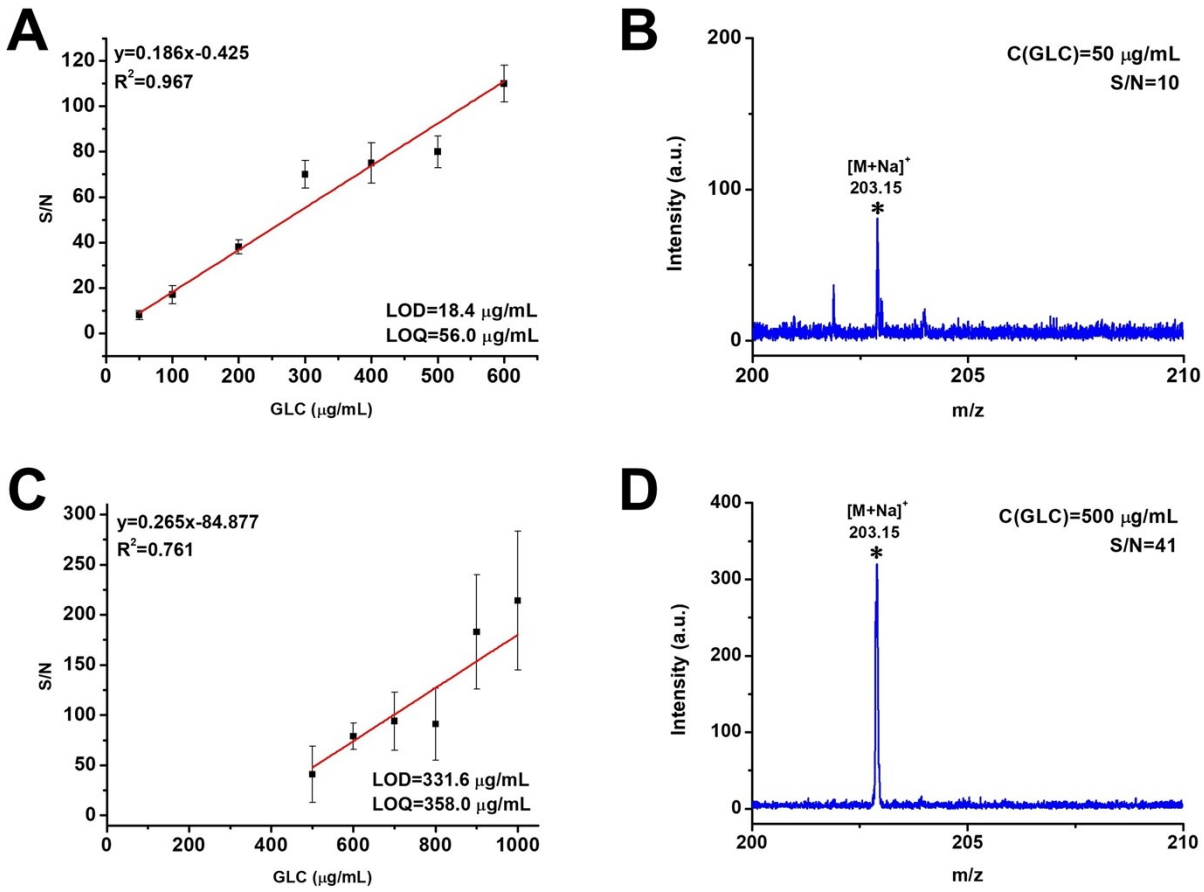


Figure S25. The cationization matrix of PTZ(A)$_2$-Ph(A) applied to the detection of urine glucose (GLC). The MALDI-TOF MS response curve used for the analysis limit-of-detection (LOD) and limit-of-quantitative (LOQ) and corresponding typical MALDI mass spectrum of urine glucose (GLC) detection in positive-ion mode with (A-B) PTZ(A)$_2$-Ph(A) matrix and (C-D) DHB matrix.

$$Glu + NaCl \rightarrow Glu \cdot Na^+ + Cl^- \quad (1)$$

$\Delta H = -88.70\ kcal/mol$

$\Delta G = -91.86\ kcal/mol$

$$\left[PTZ(A)_2 - Ph(A)\right]^{\cdot +} + Glu + NaCl \rightarrow \left[PTZ(A)_2 - Ph(A)\right]^{\cdot +} Cl^- + Glu \cdot Na^+ \quad (2)$$

$\Delta H = -12.90\ kcal/mol$

$\Delta G = -2.33\ kcal/mol$

$$\left[PXZ(A)_2 - Ph(A)\right]^{\cdot +} + Glu + NaCl \rightarrow \left[PXZ(A)_2 - Ph(A)\right]^{\cdot +} Cl^- + Glu \cdot Na^+ \quad (3)$$

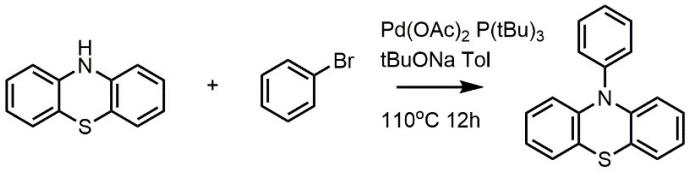
$\Delta H = -10.36\ kcal/mol$

$\Delta G = 0.20\ kcal/mol$

Scheme S2. The calculated enthalpy changes (*ΔH*) and Gibbs free energy changes (*ΔG*) of the reactions related to the cationization of GLC.

Supporting Data Set 1

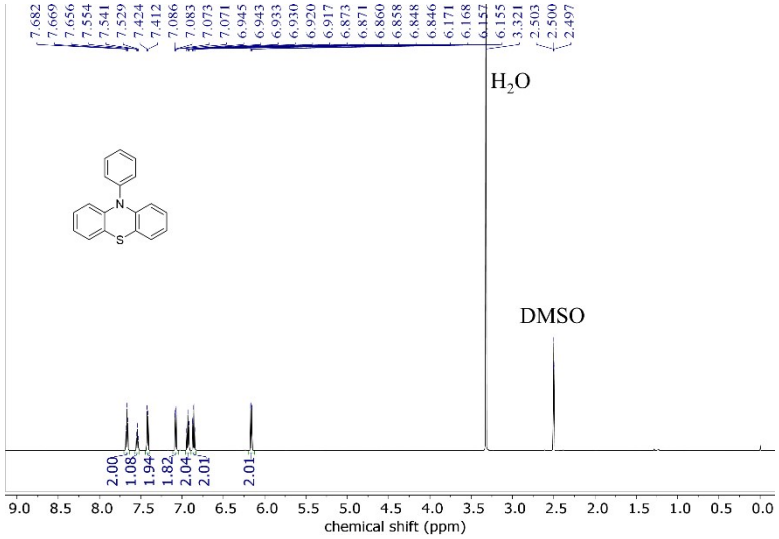
1. Synthesis of 10-phenyl-10H-phenothiazine (PTZ-Ph)



A mixture of 10*H*-phenothiazine (0.797 g, 4.0 mmol), 4-bromobenzonitrile (0.691 g, 4.4 mmol), sodium tert-butoxide (1.153 g, 12.0 mmol), catalyst Pd $(OAc)_2$ (90 mg, 10 mol%), and ligand P(*t*Bu)$_3$ (1.8 mL, 10 wt % in toluene) in toluene (40 mL) was degassed by three freeze–pump–thaw cycles, and then heated at 110 ^{o}C for 12 hours under nitrogen atmosphere. After cooling to room temperature, the mixture was extracted with DCM. The organic phase was collected and dried by anhydrous $MgSO_4$. The organic solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a white solid in a yield of 73%. The spectral data matched those previously reported[2].

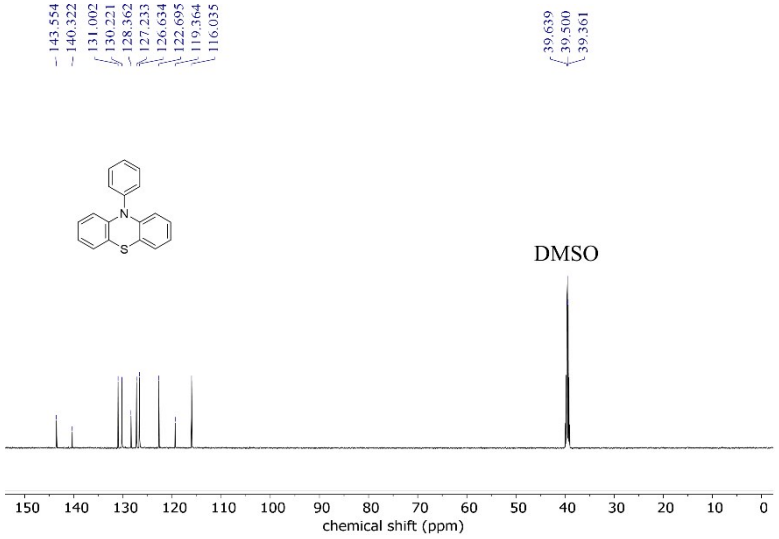
[1] H NMR spectrum of 10-phenyl-10H- phenothiazine (PTZ-Ph)

^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ = 7.67 (t, *J* = 7.8 Hz, 2H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.08 (dd, *J* = 7.6, 1.4 Hz, 2H), 6.93 (td, 2H), 6.86 (td, *J* = 7.4, 1.3 Hz, 2H), 6.16 (dd, *J* = 8.2, 1.2 Hz, 2H).

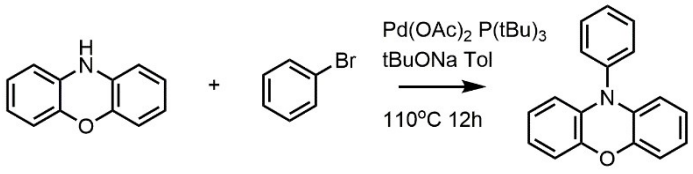


[13] C NMR spectrum of 10-phenyl-10H- phenothiazine (PTZ-Ph)

^{13}C NMR (151 MHz, DMSO-d_6, ppm): δ = 143.55, 140.32, 131.00, 130.22, 128.36, 127.23, 126.63, 122.70, 119.36, 116.04, 39.64, 39.50, 39.36.



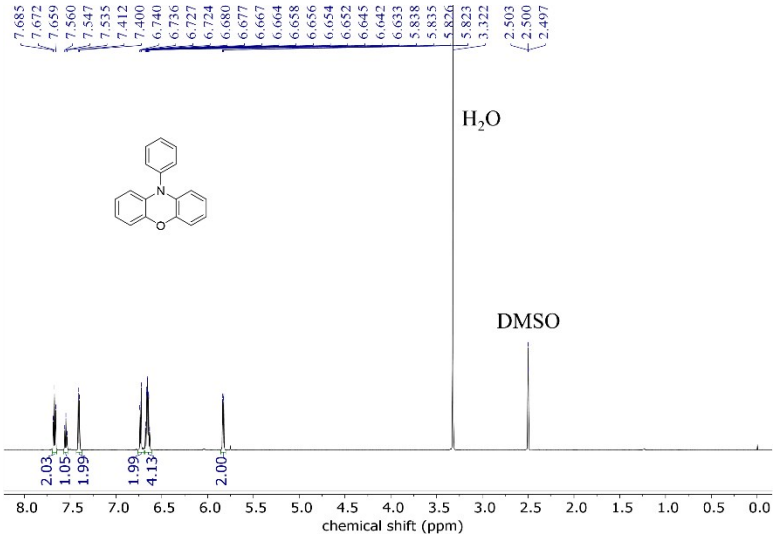
2. Synthesis of 10-phenyl-10H-phenoxazine (PXZ-Ph)



A mixture of 10*H*-phenoxazine (0.733 g, 4.0 mmol), 4-bromobenzonitrile (0.691 g, 4.4 mmol), sodium tert-butoxide (1.153 g, 12.0 mmol), catalyst Pd $(OAc)_2$ (90 mg, 10 mol%), and ligand P(*t*Bu)$_3$ (1.8 mL, 10 wt% in toluene) in toluene (40 mL) was degassed by three freeze–pump–thaw cycles, and then heated at 110 ^{o}C for 12 hours under nitrogen atmosphere. After cooling to room temperature, the mixture was extracted with DCM. The organic phase was collected and dried by anhydrous $MgSO_4$. The organic solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a white solid in a yield of 73 %.

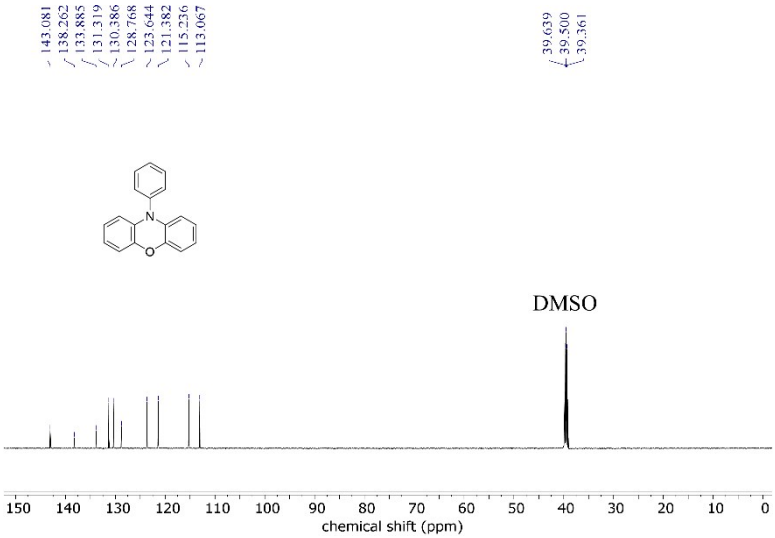
[1] H NMR spectrum of 10-phenyl-10H-phenoxazine (PXZ-Ph)

^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ = 7.64 (t, *J* = 7.8 Hz, 2H), 7.52 (t, *J* = 7.5 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 2H), 6.70 (dd, *J* = 7.5, 1.9 Hz, 2H), 6.66 – 6.60 (m, 4H), 5.80 (dd, *J* = 7.5, 1.9 Hz, 2H).



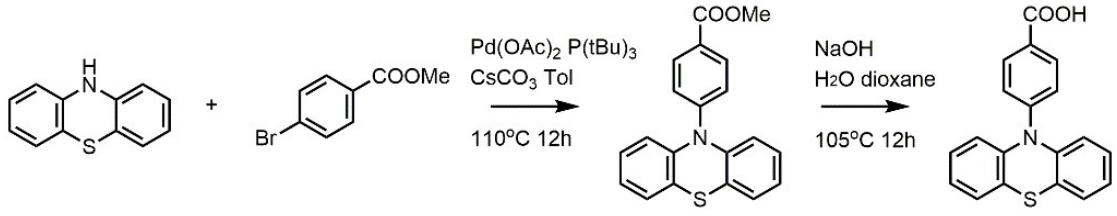
[13] C NMR spectrum of 10-phenyl-10H-phenoxazine (PXZ-Ph)

^{13}C NMR (151 MHz, DMSO-d_6, ppm): δ =143.08, 138.26, 133.89, 131.32, 130.39, 128.77, 123.64, 121.38, 115.24, 113.07, 39.64, 39.50, 39.36.



Supporting Data Set 2

3. Synthesis of 4-(10H-phenothiazin-10-yl) benzoic acid (PTZ-Ph(A))

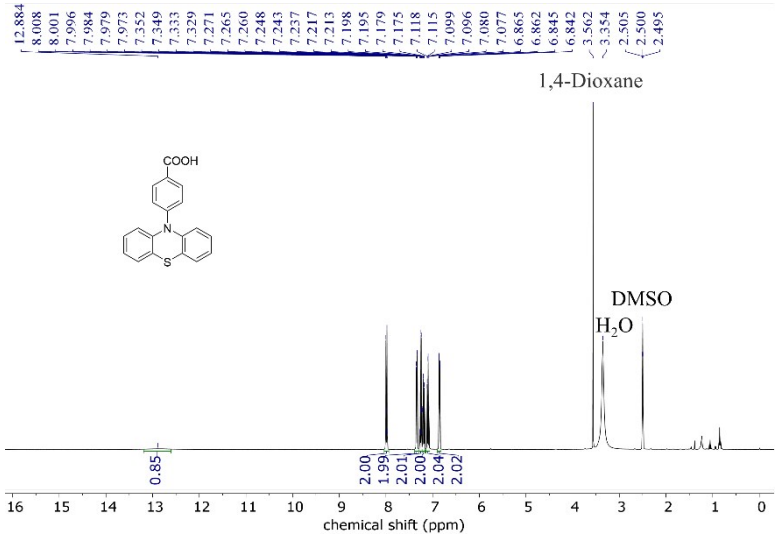


A mixture of 10*H*-phenothiazine (0.399 g, 2.0 mmol), methyl 4-bromobenzoate (0.473 g, 2.2 mmol), $CsCO_3$ (4.955 g, 6.0 mmol), catalyst $Pd(OAc)_2$ (45 mg, 10 mol%), and ligand P(*t*Bu)$_3$ (0.9 mL, 10 wt% in toluene) in toluene (40 mL) was degassed by three freeze–pump–thaw cycles, and then heated at 110 ^oC for 12 hours under nitrogen atmosphere. After cooling to room temperature, the mixture was extracted with DCM. The organic phase was collected and dried by anhydrous $MgSO_4$. The organic solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose yellow solid in a yield of 77%.

To a solution of methyl 4-(10H-phenothiazin-10-yl) benzoate (0.333 g, 1.0 mmol) in 20 mL of dioxane, a solution of NaOH (0.320 g, 8 mmol) in H_2O (10 mL) was added, then stirred under argon atmosphere at 105 ^oC for 12h. After cooling down to room temperature, the concentrated HCl aq. solution (37 %, 10 mL) was added into the mixture and the precipitate was filtered and washed with water and hexane to give the pure product in 96% yield.

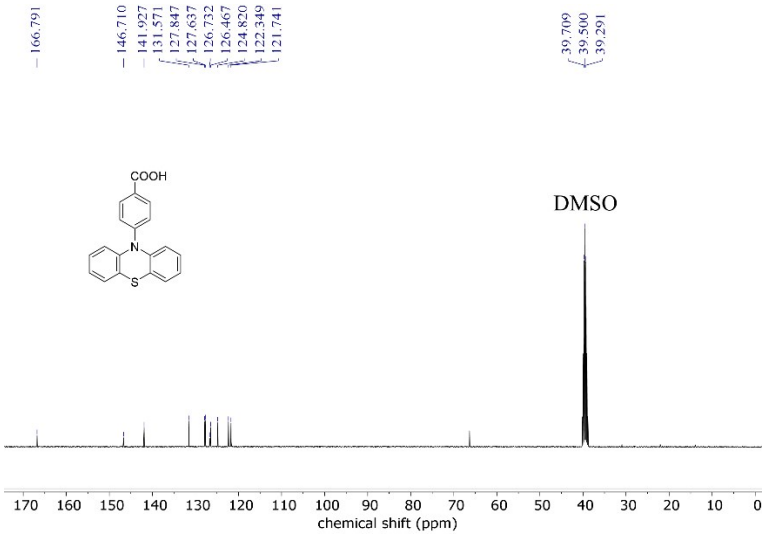
[1] H NMR spectrum of 4-(10H-phenothiazin-10-yl) benzoic acid (PTZ-Ph(A))

[1]H NMR (400 MHz, DMSO-d_6, ppm): δ = 12.88 (s, 1H), 7.99 (d, *J* = 8.8 Hz, 2H), 7.34 (dd, *J* = 7.6, 1.5 Hz, 2H), 7.20 (td, *J* = 7.4, 1.2 Hz, 2H), 7.10 (td, *J* = 7.5, 1.3 Hz, 2H), 6.85 (dd, *J* = 8.1, 1.1 Hz, 2H).

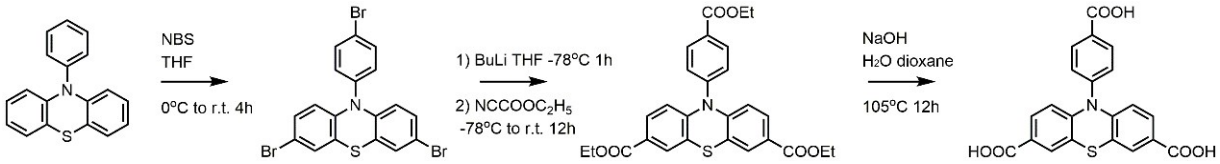


[13] C NMR spectrum of 4-(10H-phenothiazin-10-yl) benzoic acid (PTZ-Ph(A))

[13]C NMR (101 MHz, DMSO-d_6, ppm): δ = 166.79, 146.71, 141.93, 131.57, 127.85, 127.64, 126.73, 126.47, 124.82, 122.35, 121.74, 39.71, 39.50, 39.29.



4. Synthesis of 10-(4-carboxyphenyl)-10H-phenothiazine-3,7-dicarboxylic acid ($PTZ(A)_2$-Ph(A))

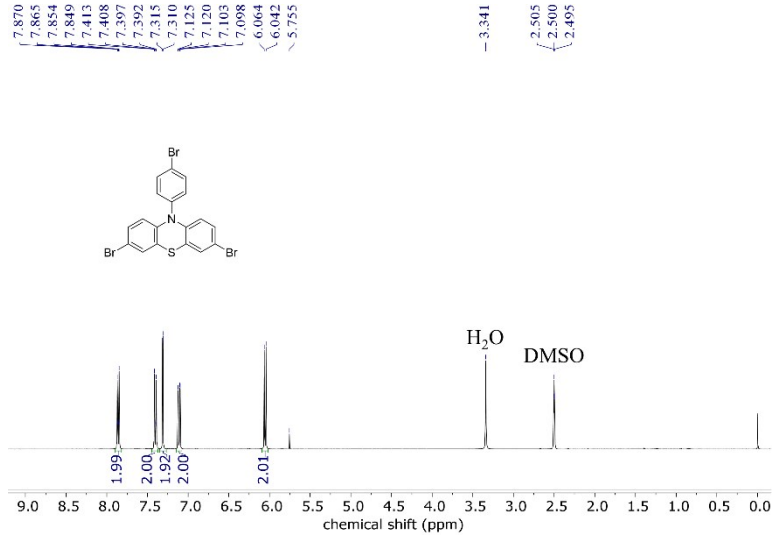


4.1 Synthesis of 3,7-dibromo-10-(4-bromophenyl)-10H-phenothiazine

To a solution of PTZ-Ph (0.827 g, 3.0 mmol) in 20 mL of tetrahydrofuran (THF), a solution of N-bromosuccinimide (1.922 g, 10.8 mmol) in THF (10 mL) was added dropwise at 0 °C, then stirred under argon atmosphere at room temperature for a period of 4 h. After the reaction, the mixture was extracted with DCM three times. The combined organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose white solid in a yield of 70 %.

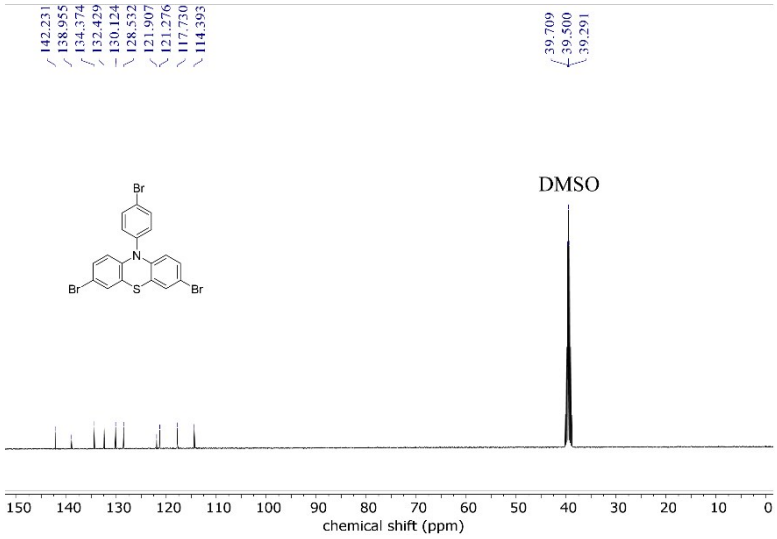
[1] H NMR spectrum of 3,7-dibromo-10-(4-bromophenyl)-10H-phenothiazine

[1]H NMR (400 MHz, DMSO-d_6, ppm): δ = 7.86 (d, *J* = 8.7 Hz, 2H), 7.41 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 2.3 Hz, 2H), 7.12 (dd, *J* = 8.8, 2.3 Hz, 2H), 6.06 (d, *J* = 8.8 Hz, 2H).



[13] C NMR spectrum of 3,7-dibromo-10-(4-bromophenyl)-10H-phenothiazine

[13]C NMR (101 MHz, DMSO-d_6, ppm): δ = 142.23, 138.96, 134.37, 132.43, 130.12, 128.53, 121.91, 121.28, 117.73, 114.39, 39.71, 39.50, 39.29.

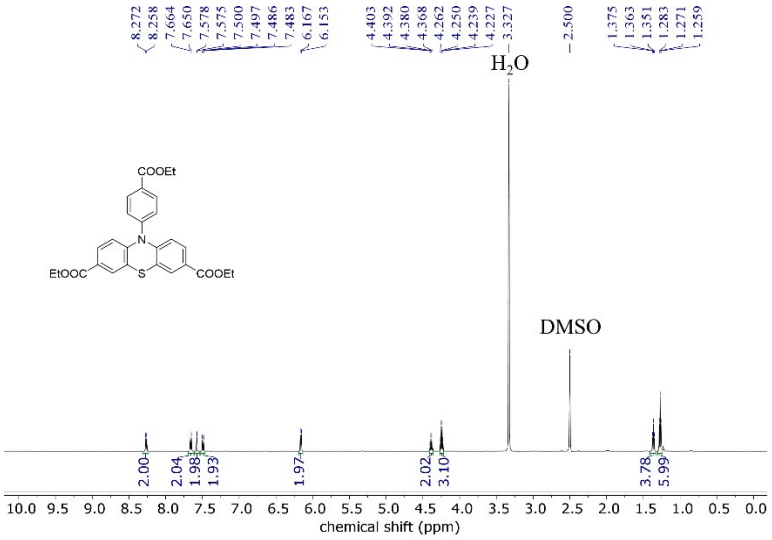


4.2. Synthesis of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenothiazine-3,7-dicarboxylate

To a suspension of 1.024 g (2.0 mmol) of 3,7-dibromo-10-(4-bromophenyl)-10H-phenothiazine in 20 mL of anhydrous THF at -78 ^{o}C, a portion of 2.9 mL of n-BuLi (7.2 mmol, 2.5 M solution in n-hexane) was slowly added, and the reaction mixture was stirred for 1 hour. The $NCCOOC_2H_5$ (9 mmol, 0.9 mL) was added to above reaction mixture at same temperature, then stirred under argon atmosphere at room temperature overnight. After the reaction, the reaction mixture was poured into water and extracted with DCM. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose yellow solid in a yield of 55 %.

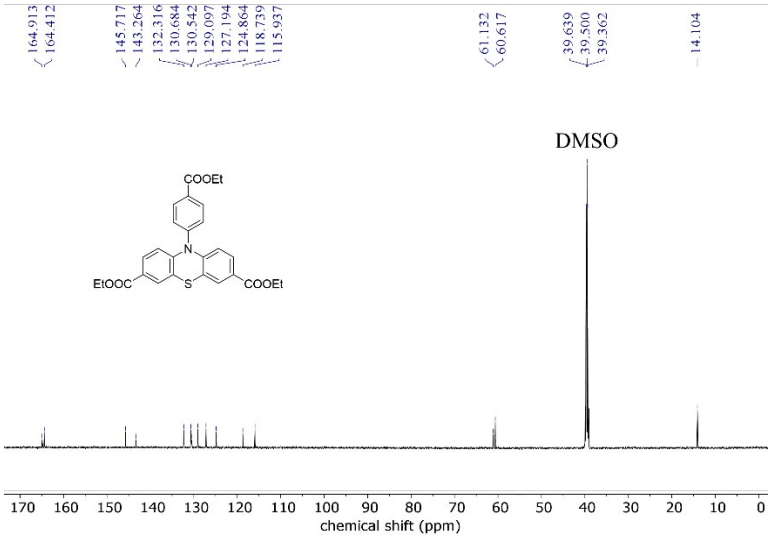
^{1}H NMR spectrum of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenothiazine-3,7-dicarboxylate

^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ = 8.24 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 1.6 Hz, 2H), 7.46 (dd, *J* = 8.7, 1.8 Hz, 2H), 6.13 (d, *J* = 8.7 Hz, 2H), 4.36 (d, *J* = 7.1 Hz, 2H), 4.22 (q, *J* = 7.1 Hz, 3H), 1.34 (t, *J* = 7.1 Hz, 4H), 1.24 (t, *J* = 7.1 Hz, 6H).



^{13}C NMR spectrum of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenothiazine-3,7-dicarboxylate

^{13}C NMR (151 MHz, DMSO-d_6, ppm): δ = 164.91, 164.41, 145.72, 143.26, 132.32, 130.68, 130.54, 129.10, 127.19, 124.86, 118.74, 115.94, 61.13, 60.62, 39.64, 39.50, 39.36, 14.10.

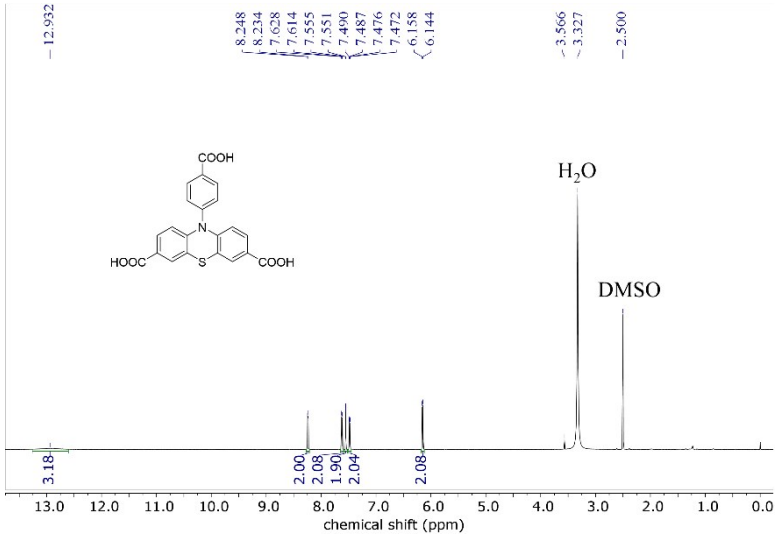


4.3 Synthesis of 10-(4-carboxyphenyl)-10H-phenothiazine-3,7-dicarboxylic acid ($PTZ(A)_2$-Ph(A))

To a solution of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenothiazine-3,7-dicarboxylate (0.246 g, 0.5 mmol) in 20 mL of dioxane, a solution of NaOH (0.160 g, 4 mmol) in H_2O (10 mL) was added, then stirred under argon atmosphere at 105 ^oC for 12 h. After cooling down to room temperature, the concentrated HCl aq. solution (37 %, 10 mL) was added into the mixture and the precipitate was filtered and washed with water and hexane to give the pure product in 95 % yield.

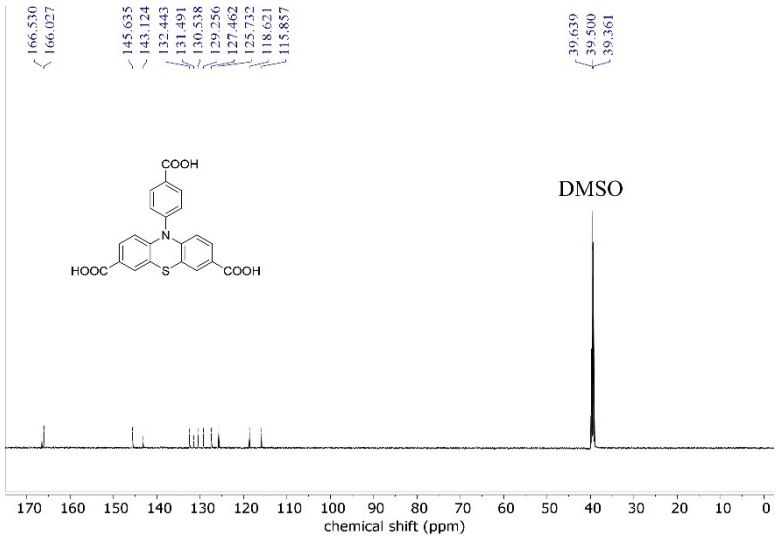
^{1}H NMR spectrum of 10-(4-carboxyphenyl)-10H-phenothiazine-3,7-dicarboxylic acid (PTZ(A)$_2$-Ph(A))

^{1}H NMR (600 MHz, DMSO-d_6, ppm): δ = 12.90 (s, 3H), 8.21 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.53 (d, *J* = 2.0 Hz, 2H), 7.45 (dd, *J* = 8.7, 2.0 Hz, 2H), 6.12 (d, *J* = 8.7 Hz, 2H).

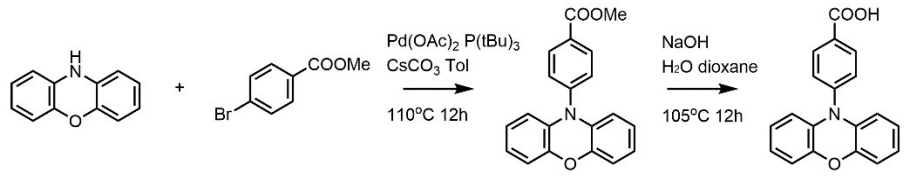


^{13}C NMR spectrum of 10-(4-carboxyphenyl)-10H-phenothiazine-3,7-dicarboxylic acid (PTZ(A)$_2$-Ph(A))

^{13}C NMR (151 MHz, DMSO-d_6, ppm); δ = 166.53, 166.03, 145.64, 143.12, 132.44, 131.49, 130.54, 129.26, 127.46, 125.73, 118.62, 115.86, 39.64, 39.50, 39.36.



5. Synthesis of 4-(10H-phenoxazine-10-yl) benzoic acid (PXZ-Ph(A))

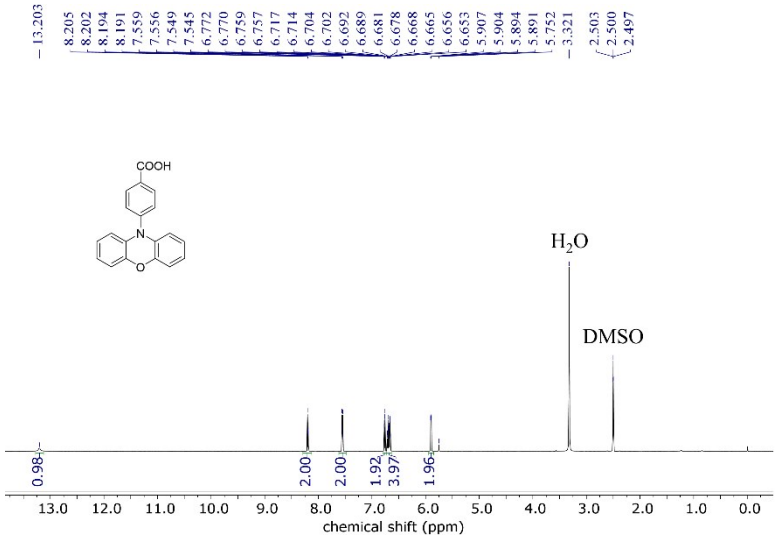


A mixture of 10*H*-phenoxazine (0.366 g, 2.0 mmol), methyl 4-bromobenzoate (0.473 g, 2.2 mmol), $CsCO_3$ (4.955 g, 6.0 mmol), catalyst $Pd(OAc)_2$ (45 mg, 10 mol%), and ligand $P(tBu)_3$ (0.9 mL, 10 wt% in toluene) in toluene (40 mL) was degassed by three freeze–pump–thaw cycles, and then heated at 110 ^oC for 12 hours under nitrogen atmosphere. After cooling to room temperature, the mixture was extracted with DCM. The organic phase was collected and dried by anhydrous $MgSO_4$. The organic solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose yellow solid in a yield of 77 %.

To a solution of methyl 4-(10*H*-phenoxazine -10-yl) benzoate (0.317 g, 1.0mmol) in 20 mL of dioxane, a solution of NaOH (0.320 g, 8 mmol) in H_2O (10 mL) was added, then stirred under argon atmosphere at 105 ^oC for 12 h. After cooling down to room temperature, the concentrated HCl aq. solution (37 %, 10 mL) was added into the mixture and the precipitate was filtered and washed with water and hexane to give the pure product in 96 % yield.

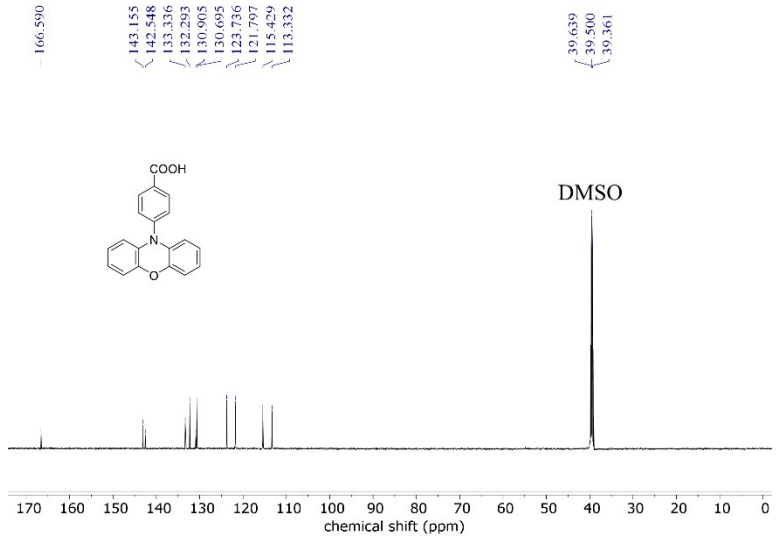
[1] H NMR spectrum of 4-(10H-phenoxazine-10-yl) benzoic acid (PXZ-Ph(A))

^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ = 13.18 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 8.4 Hz, 2H), 6.74 (dd, *J* = 7.7, 1.6 Hz, 2H), 6.66 (dtd, *J* = 21.7, 7.5, 1.6 Hz, 4H), 5.87 (dd, *J* = 7.8, 1.6 Hz, 2H).

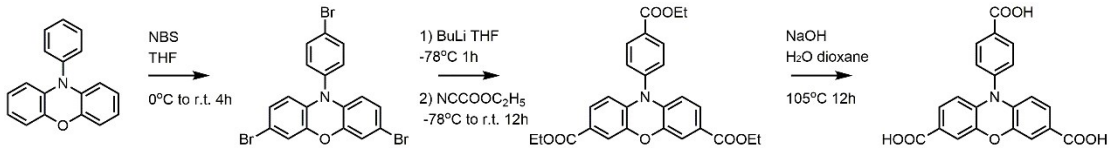


[13] C NMR spectrum of 4-(10H-phenoxazine-10-yl) benzoic acid (PXZ-Ph(A))

^{13}C NMR (151 MHz, DMSO-d_6, ppm): δ = 166.59, 143.15, 142.55, 133.34, 132.29, 130.90, 130.70, 123.74, 121.80, 115.43, 113.33, 39.64, 39.50, 39.36.



6. Synthesis of 10-(4-carboxyphenyl)-10H-phenoxazine -3,7-dicarboxylic acid ($PXZ(A)_2$-Ph(A))

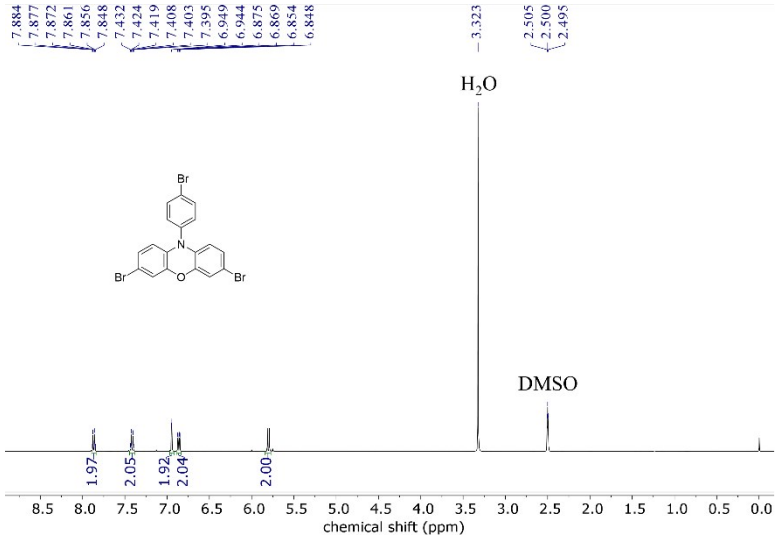


6.1 Synthesis of 3,7-dibromo-10-(4-bromophenyl)-10H-phenoxazine

To a solution of PXZ-Ph (0.778 g, 3.0 mmol) in 20 mL of tetrahydrofuran (THF), a solution of N-bromosuccinimide (1.922 g, 10.8 mmol) in THF (10 mL) was added dropwise at 0 °C, then stirred under argon atmosphere at room temperature for a period of 4 h. After the reaction, the mixture was extracted with DCM three times. The combined organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose white solid in a yield of 70 %.

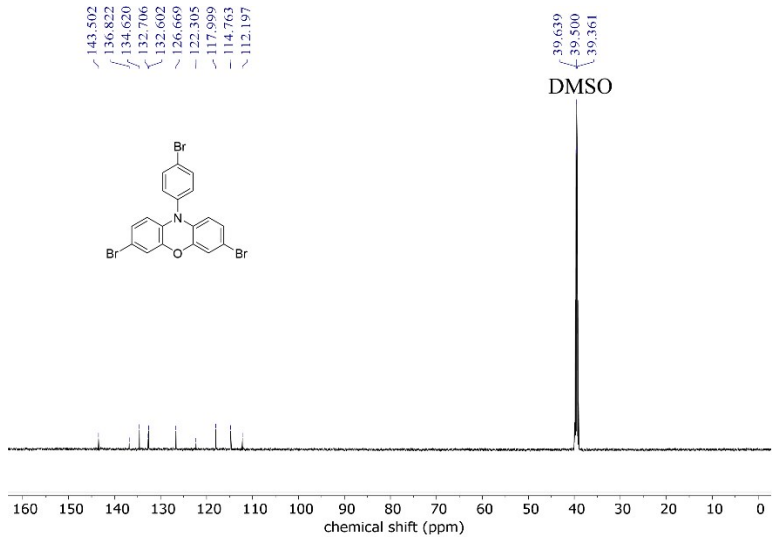
[1] H NMR spectrum of 3,7-dibromo-10-(4-bromophenyl)-10H-phenoxazine

[1]H NMR (400 MHz, DMSO-d_6, ppm): δ = 7.87 (td, 2H), 7.42 (td, 2H), 6.95 (d, *J* = 2.2 Hz, 2H), 6.86 (dd, *J* = 8.6, 2.2 Hz, 2H), 5.80 (d, *J* = 8.6 Hz, 2H).



[13] C NMR spectrum of 3,7-dibromo-10-(4-bromophenyl)-10H-phenoxazine

[13]C NMR (151 MHz, DMSO-d_6, ppm): δ = 143.50, 136.82, 134.62, 132.71, 132.60, 126.67, 122.30, 118.00, 114.76, 112.20, 39.64, 39.50, 39.36.

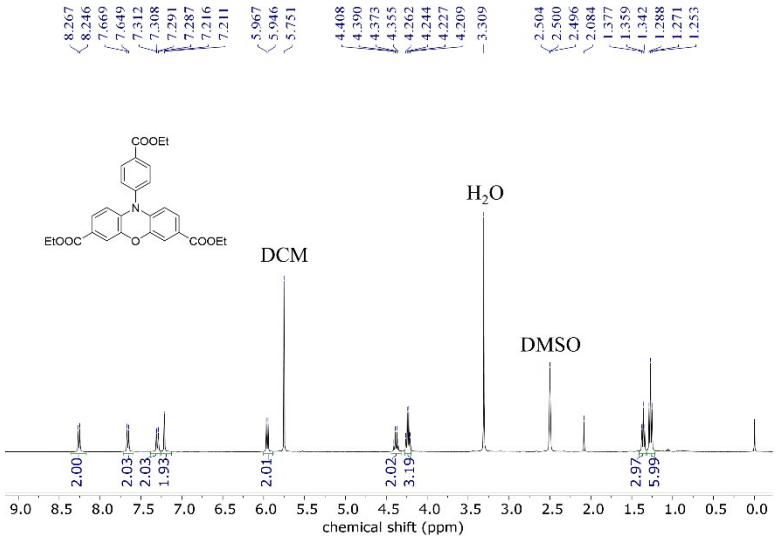


6.2. Synthesis of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenoxazine-3,7-dicarboxylate

To a suspension of 0.992 g (2.0 mmol) of 3,7-dibromo-10-(4-bromophenyl)-10*H*-phenoxazine in 20 mL of anhydrous THF at -78 ^{o}C, a portion of 2.9 mL of n-BuLi (7.2 mmol, 2.5 M solution in n-hexane) was slowly added, and the reaction mixture was stirred for 1 hour. The $NCCOOC_2H_5$ (9 mmol, 0.9 mL) was added to above reaction mixture at same temperature, then stirred under argon atmosphere at room temperature overnight. After the reaction, the reaction mixture was poured into water and extracted with DCM. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel to afford a primrose yellow solid in a yield of 55 %.

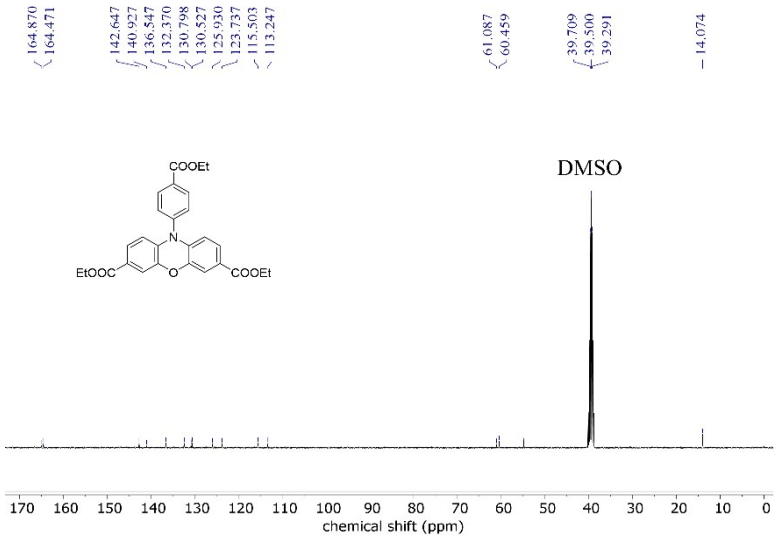
^{1}H NMR spectrum of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenoxazine-3,7-dicarboxylate

^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.26 (d, *J* = 8.4 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.30 (dd, *J* = 8.4, 1.8 Hz, 2H), 7.21 (d, *J* = 1.8 Hz, 2H), 5.96 (d, *J* = 8.4 Hz, 2H), 4.38 (q, *J* = 7.1 Hz, 2H), 4.24 (q, *J* = 7.1 Hz, 4H), 1.36 (t, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 6H).



^{13}C NMR spectrum of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10H-phenoxazine-3,7-dicarboxylate

^{13}C NMR (101 MHz, DMSO-d_6, ppm): δ = 164.87, 164.47, 142.65, 140.93, 136.55, 132.37, 130.80, 130.53, 125.93, 123.74, 115.50, 113.25, 61.09, 60.46, 39.71, 39.50, 39.29, 14.07.

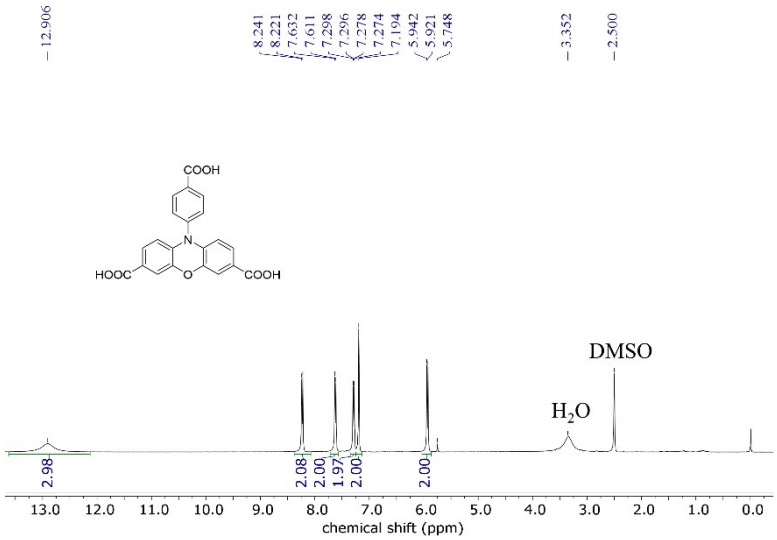


6.3 Synthesis of 10-(4-carboxyphenyl)-10H-phenoxazine -3,7-dicarboxylic acid ($PXZ(A)_2$-Ph(A))

To a solution of diethyl 10-(4-(ethoxycarbonyl) phenyl)-10*H*-phenoxazine-3,7-dicarboxylate (0.238 g, 0.5 mmol) in 20 mL of dioxane, a solution of NaOH (0.160 g, 4 mmol) in H_2O (10 mL) was added, then stirred under argon atmosphere at 105 ^oC for 12 h. After cooling down to room temperature, the concentrated HCl aq. solution (37 %, 10 mL) was added into the mixture and the precipitate was filtered and washed with water and hexane to give the pure product in 95 % yield.

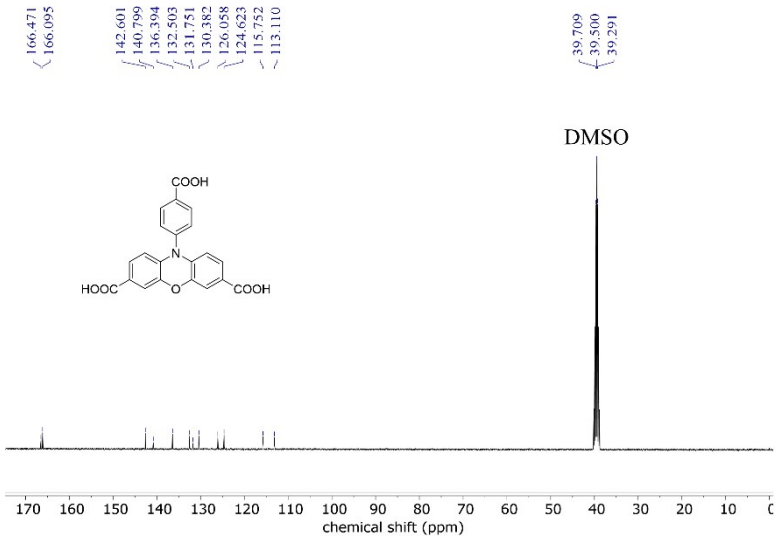
[1] H NMR spectrum of 10-(4-carboxyphenyl)-10H-phenoxazine -3,7-dicarboxylic acid (PXZ(A)$_2$-Ph(A))

[1]H NMR (400 MHz, DMSO-d_6, ppm): δ = 12.91 (s, 3H), 8.24 (d, *J* = 8.3 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.20 (s, 2H), 5.94 (d, *J* = 8.4 Hz, 2H).



[13]C NMR spectrum of 10-(4-carboxyphenyl)-10H-phenoxazine -3,7-dicarboxylic acid (PXZ(A)$_2$-Ph(A))

[13]C NMR (101 MHz, DMSO-d_6, ppm): δ = 166.47, 166.10, 142.60, 140.80, 136.39, 132.50, 131.75, 130.38, 126.06, 124.62, 115.75, 113.11, 39.71, 39.50, 39.29.



Reference

[1] M. J. Frisch, G. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. Sonnenberg, M. Hada and D. Fox, 2009.

[2] N. J. Treat, H. Sprafke, J. W. Kramer, P. G. Clark, B. E. Barton, J. Read de Alaniz, B. P. Fors and C. J. Hawker, *Journal of the American Chemical Society*, 2014, **136**, 16096-16101.

Abbreviations:

PTZ-Ph: 10-phenyl-10H-phenothiazine

PXZ-Ph: 10-phenyl-10H-phenoxazine

PTZ-Ph(A): 4-(10H-phenothiazin-10-yl) benzoic acid

PXZ-Ph(A): 4-(10H-phenoxazine-10-yl) benzoic acid

$PTZ(A)_2$-Ph(A): 10-(4-carboxyphenyl)-10H-phenothiazine-3,7-dicarboxylic acid

$PXZ(A)_2$-Ph(A): 10-(4-carboxyphenyl)-10H-phenoxazine-3,7-dicarboxylic acid